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THE PRESENCE OF AN INSULIN-LIKE SUBSTANCE IN GASTRIC AND DUODENAL MUCOSA AND ITS RELATION TO GASTRIC SECRETION

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Ivy, S. A. Matthews, Jacoby and Vloedman (1) working together during the fall of 1922, found a blood sugar reducing substance in "gastrin" prepared by the Koch method and in "secretin" prepared by the Bayliss and Starling method. The active preparations lost their activity at the end of two months. Other fresh preparations of "gastrin" and "secretin" were made which stimulated gastric and pancreatic secretion but failed to reduce blood sugar. In view of these results we felt certain that some blood sugar reducing substance was present in the duodenal and gastric mucosa, but that the "gastrin" and "secretin" methods of extraction could not be relied upon to consistently demonstrate its presence.

It was then decided to extract the gastric and duodenal mucosa using a method known to yield insulin when applied to pancreatic and other tissues. The Shaffer method as modified by Fisher (2) was used. This proved to be satisfactory as an insulin-like substance was isolated from the gastric and duodenal mucosa.

Besides the above question of the presence of insulin in the gastric and duodenal mucosa, we were interested in the following questions: *a*, Will insulin stimulate the secretion of gastric juice? *b*. Is insulin hypoglycemia incompatible with gastric secretion excited by a meal? *c*, Can a gastric "secretin" be extracted by the Shaffer-Fisher method for insulin?

METHOD. The method of extraction was as follows: To one kilogram of fresh hog's gastric or duodenal mucosa were added 1200 cc. of 95 per cent alcohol, 300 cc. of water and 40 cc. of concentrated HCl. On returning to the laboratory the tissue was removed from the solution, ground

and frozen for 24 hours. At the end of this time it was returned to the original acid-water-alcohol solution and allowed to stand for 12 hours. The liquid was then poured off and the tissue re-extracted with 100 cc. of 50 to 60 per cent alcohol for 12 hours. The two liquids were then combined and the tissue residue was discarded. The mixture of the two liquids was then exposed to a current of hot air to evaporate the alcohol. The remaining acid-water mixture was half saturated with ammonium sulphate. The filtrate was evaporated to dryness and the ammonium sulphate was removed from the residue by dialysis through a collodion tube. The water passing into the collodion tube made a solution of the dried residue. This solution, which we call preparation I, was then concentrated to desired volume for injection. The flocculent precipitate resulting from half saturation with ammonium sulphate was dissolved in 100 cc. of 70 per cent alcohol, centrifuged and the supernatant fluid was decanted. To this fluid one volume of 95 per cent alcohol was added. The precipitate was collected by filtration, dried and dissolved in water, which solution we call preparation II. The filtrate was further precipitated with eight volumes of 95 per cent alcohol. The precipitate (insulin) was collected by filtration and dissolved in water, which solution we call preparation III. The filtrate was evaporated to dryness and the residue dissolved in water, which solution we call preparation IV.

Results. *Presence of an insulin-like substance in "gastrin" and "secretin" preparations:* Fresh hogs' gastric and duodenal mucosa was extracted by the Koch method for "gastrin." "Secretin" was made by the Bayliss and Starling method from fresh hogs' duodenal mucosa and concentrated ten times under reduced pressure at 40°C.

The subcutaneous injection of from 4 to 6 cc. of these extracts in dogs starved 48 hours caused a hypoglycemia, as shown in figure 1. Sometimes the blood sugar would rise slightly before falling one hour after injection. The activity of these preparations disappeared in two months. Three other sets of preparations were made with the same procedure which either had no effect on blood sugar or caused it to rise slightly.

Presence of insulin in gastric and duodenal mucosa: Fresh hogs' gastric and duodenal mucosa was extracted for insulin by the method given above, the Shaffer-Fisher method.

When this extract (preparation III) was injected subcutaneously in rabbits a reduction of blood sugar occurred, as is shown in figure 2. The extract from one hog's gastric mucosa was required to produce a definite reduction, which shows that the blood sugar reducing substance is only one-fiftieth as concentrated as it is in cattle's pancreas.

When the extract of one hog's gastric mucosa was injected into a normal 12-kilo dog starved 18 hours, the blood sugar was definitely lowered, e.g., from 0.100 to 0.071 in 4 hours.

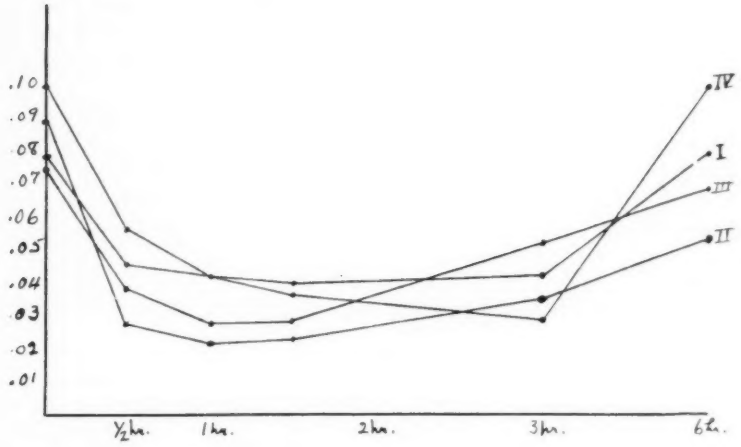

Fig. 1. Dogs starved 48 hours .

I, Weight 6 kilos. Injected subcutaneously 4 cc. "secretion" by "gastrin" method.

II, Weight 8 kilos. Injected subcutaneously 4 cc. concentrated Bayliss and Starling "secretin."

III, Weight 8 kilos. Injected subcutaneously 4 cc. "gastrin."

IV, Weight 10 kilos. Injected subcutaneously 5 cc. "gastrin."

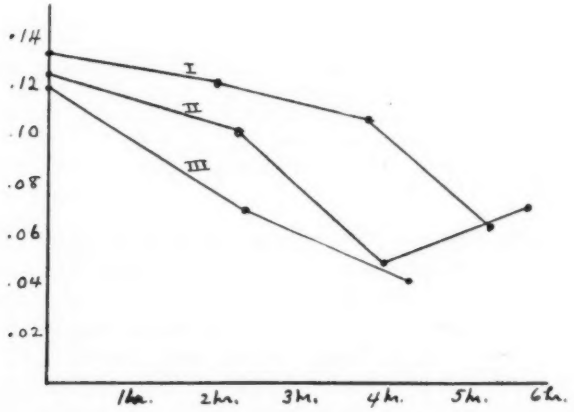

Fig. 2. Rabbit I, 3 kgm., starved 24 hours. Injected "insulin" from 1 hog's stomach.

Rabbit II, 3 kgn., not starved. Injected "insulin" from 2 hog's stomachs.

Rabbit III, 3 kgm., starved 24 hours. Injected "insulin" from 1 hog's stomach.

We have used the extract on one pancreatectomized dog with the result that the blood sugar was reduced from 0.444 to 0.285, the extract of two hogs' gastric mucosa being used. Similar results were obtained with the extracts of duodenal mucosa.

Does insulin excite gastric secretion? The observations of Ivy, S. A. Matthews et al showed that "gastrin" and insulin are not identical substances, because the blood sugar reducing activity of the "gastrin" preparation disappeared while the "gastrin" activity remained. Even though they are not identical, they both may excite gastric secretion.

TABLE 1

Showing that insulin does not stimulate gastric secretion and does not inhibit gastric secretion excited by a meal

Pavlov pouch dog

PROCEDURE	TIME O'CLOCK	GASTRIN SECRETION			REMARKS
		Amount	Free acidity*	Total acidity*	
		cc.			
Continuous	10–11	1.7	25	57	
Injected insulin: 2 cc. at 11:00	11–12	1.8	22	37	Cattle's pancreas
	12–1	1.1	15	25	
	1–2	0.5	0	10	
Meal of meat at 2:00	2–3	2.7	50	62	3:30 animal comatose blood sugar 0.019
	3–4	6.5	80	92	
	4–5	4.7	82	95	4:30 animal comatose

3:30 animal comatose with muscular twitchings. Gave 5 grams glucose intravenously which resulted in recovery.

4:30 animal comatose again. Gave 5 grams glucose intravenously with recovery. Also gave 20 grams glucose by mouth.

7:30 Animal depressed. Again administered glucose.

*Clinical units.

When insulin extracted from cattle's pancreas or from hog's gastric mucosa was injected subcutaneously in Pavlov-pouch dogs gastric secretion was not excited (table 1).

Is insulin hypoglycemia incompatible with gastric secretion excited by a meal? Two dogs with a Pavlov pouch were injected with sufficient insulin to cause hypoglycemic symptoms. Then an hour or two after the injection of insulin, a meal was fed. To our surprise the normal secretory response to a meal occurred in spite of the marked hypoglycemic symptoms observed. A typical result is shown in table 1.

Can a gastric secretin be extracted by the Shaffer-Fisher method? In order to answer this question it was necessary for us to put to test all

fractions. We have already stated that preparation III (insulin) does not excite gastric secretion in Pavlov pouch dogs. We also found that preparation II was negative. Preparation I, the acid-water fraction after precipitation by half saturation with ammonium sulphate, is decidedly positive, as is shown by the results in table 2. This is a clear light straw colored solution less irritating than "gastrin." The Koch method yields approximately ten times more gastrin than this method. Preparation IV contains a trace of "gastrin" only, as very slight stimulation occurred when it was injected.

Preparation II not toxic for rats. Fisher (2) has shown that preparation II when cattle's pancreas is extracted is very toxic when injected

TABLE 2

Showing that gastric secretion is present in the acid-water filtrate after precipitation by half saturation with ammonium sulphate. Preparation I

Pavlov pouch dog

PROCEDURE	TIME O'CLOCK	GASTRIC JUICE			REMARKS
		Amount	Free acidity*	Total acidity*	
		cc.			
Continuous secretion	2–3	1.7	0	7	
Inject 6 cc. Prep. I.	3–4	7.8	45	62	Acid-water fraction
	4–5	1.2	47	65	
A second experiment					
Continuous secretion	3–4	2.0	0	20	
Inject 6 cc. Prep. I.	4–5	5.4	70	92	Acid-water fraction
	5–6	2.0	75	100	
	6–7	1.5	60	70	

*Clinical units.

into rats. But we have found that this preparation when hog's gastric mucosa is extracted is not toxic when injected into rats. We also failed to get abscesses or any reaction when any of the preparations were injected subcutaneously.

CONCLUSIONS

1. An insulin-like blood sugar reducing substance is present in hogs' gastric and duodenal mucosa, which has the therapeutic action of insulin on a diabetic dog. From 50 to 75 times more insulin can be extracted from cattle's pancreas than from hogs' gastric mucosa.

2. Insulin and "gastrin" are not identical substances.

3. Insulin does not excite gastric secretion; neither does it inhibit the gastric secretion excited by a meal, a blood sugar concentration of 0.019 being compatible with the formation of normal gastric juice.

4. It is possible to extract a gastric secretin from hogs' gastric and duodenal mucosa by the Shaffer-Fisher method for insulin. The gastric secretin is present in the acid-water filtrate after precipitation by half saturation with ammonium sulphate.

5. A toxic fraction similar to that obtained on extraction of cattle's pancreas is not present when hogs' gastric mucosa is extracted.

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THE PRESENCE OF "GASTRIN" IN HUMAN POST-MORTEM PYLORIC AND DUODENAL MUCOSA

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Recently Ammon and Lim (1) have extracted the human gastric mucosa by the "secretin" method and the method of Dale and Laidlaw for "gastrin." The material was obtained at postmortem from twelve to sixty-three hours after death. They used "acute methods of experimentation" to test the activity of their extracts. They concluded that the human pyloric mucosa contains either little or no gastrin, or much less gastrin than is found in lower animals.

Doubting the efficacy of the methods of the above investigators, we decided to repeat their work using Pavlov pouch dogs and the Koch method for the extraction of "gastrin."

METHODS. We have used the method outlined by Keeton and Koch (2) for preparing "gastrin" from pyloric mucosa and other tissues.

Pavlov pouch dogs have been used throughout for testing the activity of the "gastrin" preparations.

As we were not interested in a possible relation between the cause of death and the "gastrin" content of the pyloric mucosa, we put the mucosa from several stomachs together for extraction.

Preparation I was made from 200 grams of pyloric mucosa from four stomachs from the following cases:

1. 65 years old, death from peritonitis, material obtained 12 hours after death
2. 17 years old, death from myocarditis, material obtained 12 hours after death
3. 51 years old, death from myocarditis, material obtained 8 hours after death
4. 50 years old, death from cystic kidneys with abscess, material obtained 3 hours after death

The duodenal mucosa from the above cases was combined and extracted.

Preparation II was made from 220 grams of pyloric mucosa from four stomachs from the following cases:

1. 65 years old, death from endocarditis, material obtained 8 hours after death
2. 68 years old, death from surgical shock, material obtained 3 hours after death
3. 70 years old, death from nephritis, material obtained 1 hour after death
4. 52 years old, death from dilatation of stomach, material obtained 2 hours after death

The duodenal mucosa from the above cases was also combined and extracted.

Preparation III was made from 24 grams of pyloric mucosa combined with 5 grams of duodenal mucosa from a four-day-old body, cause of death unknown.

RESULTS. It was found that in all cases both the extract of the gastric and duodenal mucosa were active when injected subcutaneously in Pavlov pouch dogs. (See table 1.)

TABLE 1

Showing the activity of "gastrin" prepared from human post-mortem pyloric mucosa

Pavlov pouch dogs

PROCEDURE	TIME O'CLOCK	GASTRIC SECRETION			REMARKS
		Amount	Free acidity*	Total acidity*	
Continuous secretion.....	11–12	1.0	0	17	
Injected 2 cc. human gastrin	12–1	11.0	85	102	A very marked local reaction occurred resulting in an ulcer 3 days later
Preparation I.....	1–2	20.0	117	127	
	2–3	15.0	107	122	
	3–4	10.0	97	115	
Continuous secretion.....	10–11	1.5	17	42	
Continuous secretion.....	11–12	1.2	17	45	
Injected 1 cc. human gastrin	12–1	10.0	92	107	½ hour after injection face was very edematous and hyperemic
Preparation III.....	1–2	1.0	92	100	

*Clinical units.

When human "gastrin" was compared in activity with hogs' "gastrin," we found that preparation I was slightly more active than hogs' "gastrin," while preparation II was slightly less active. Preparation III was very similar in activity to our preparation of hogs' "gastrin." The basis of comparison was the secretory response to the extract of 5 grams of mucosa.

When preparation III was injected for the first time a marked hyperemia (dog with a white face) and edema of the face occurred, which was first noticed one-half hour after injection and lasted one and one-half hours. The edema was not due to a possible histamine effect, we believe, because when histamine was injected sufficiently to excite a similar gastric secretory response edema of the face did not occur. Marked hyperemia of the face and ears without edema occurred, however, when 1.5 mgm. of histamine were injected.

The duodenal extracts were active but only about one-half as potent as the gastric extracts.

SUMMARY

The results show that "gastrin" is present in human gastric and duodenal mucosa to the same extent as it is present in hogs' mucosa and that in the study of gastric secretion, at least, normal or physiological preparations should be used for consistent and unquestionable results.

An interesting reaction occurred in one dog, consisting of marked edema of the face, when human "gastrin" from a four-day-old body was injected.

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THE DISTRIBUTION OF VITAMINE A IN URINE AND SOME OF THE DIGESTIVE SECRETIONS

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The experiments (1) here reported were undertaken in the hope that they may throw some light on the fate of vitamine A in the body under varying states of diet and nutrition. The only body secretion in which vitamine A may serve a physiological need is milk. If this vitamine appears in the urine and the digestive secretions, this probably indicates an excess vitamine intake in the food, and the limits of storage or destruction of the vitamine in the body. It is well known that ferments and immune bodies appear in the urine and digestive secretions in proportion to their concentration in the blood. Practically nothing is known of the rate of destruction or utilization of this vitamine in the body. The storage of the vitamine is probably a question of quantity and character of the body fats.

METHOD. 1. The vitamine A content of the body fluids was determined by feeding experiments on rats. Large litters (6 to 8 young), four to five weeks old, each rat weighing 40 to 50 grams, were selected. In this way individuals from the same litter could be put on the stock diet and the several test diets, and the effects of the latter rendered more certain. The rats were taken from the mothers and put upon a theoretically adequate, synthetic diet. They were kept upon this diet for a week, ten days or two weeks, to test their growth on this diet. If the rats showed satisfactory growth, as was usually the case, we concluded that they reacted to vitamine A. Occasionally some litters did not grow, or grew only very slightly when upon this diet, even for six to eight weeks, while other litters fed from the same stock showed good growth. The litters or individuals that did not show good growth on the theoretically adequate diet were not used in further tests.

The rats were then put on the vitamine A free diet. This diet was similar to the adequate synthetic diet only that Crisco was used as the source of the fat. After ten days to two weeks upon this diet the rats ceased to gain in weight and actually lost some. We concluded that these rats were also reacting to the absence of the vitamine A. They were then put on the special test diets. If they started to gain in weight we concluded that

vitamine A was present; if they continued to lose, that it was absent. If the weight remained stationary or if the rate of loss was less than on the definitely vitamine A free food, the results are less readily interpreted. But a stationary body weight, or only very slow rate of loss would seem to indicate the presence of vitamine A, but not in sufficient quantity. Individual variation in rate of growth is a disturbing factor.

All animals used in these tests were bred in the laboratory from a large selected stock. They were fed on a diet of mixed grain, green vegetables and bread soaked in whole milk. The pregnant females were taken from the stock a few days before their young were born and put in special nesting cages. The mother and her young were kept in these smaller cages until her young were large enough to start on the experiments. In this way litters were kept separate and the exact age of each rat used was known. Experience has shown us that these young were much superior to any that might be purchased from local dealers.

Care of animals used during tests. During experiments the young animals were kept in separate cages. The cages were arranged in such a way that the bottoms consisted of fairly coarse wire netting. The bottom of each cage was supported an inch or so above a flat tray, thus allowing the feces to drop through the wire floor upon the tray. This arrangement was made after it was noticed that rats upon deficient diets would consume daily all of their own feces. Each rat was fed its special diet and watered daily. Each was weighed once each week, or twice weekly, or in certain cases as circumstances demanded, they were weighed each second day. Any rats dying during the experiment were autopsied and the gross pathology noted.

The diets. All of the diets had the following ingredients in common:

	per cent
Casein	20
Salt mixture	4
Fleischmann's yeast	5
Corn starch	46
Purified agar-agar	4

The diet high in A contained 25 per cent butter fat. The A-free diet contained 25 per cent Crisco. The special test diets contained the Crisco fat, and the extracts from the respective body fluids. In most cases the animals ate the various foods readily.

Purification of the food constituents. The casein was made from skimmed milk, following the method of Kohman (2), except that a large number of washings with ether and alcohol were used in cases where the milk contained more fat. The end point in washing the casein was considered to be reached when a small amount of the last ether filtrate showed no fat residue upon evaporation.

Much of the casein was as fine as chalk dust and very white in color. A large quantity was made before the experiments were started and this proved sufficient to carry through several entire tests. For later parts of the experiments new lots had to be made even though the technic used in purifying the casein, and even though the end point in purifying it is always the same, every worker in this field realizes that each new lot is a variable factor in animal feeding experiments.

Salt mixture. We used the salt mixture (3) proved by many previous workers to supply the inorganic needs of rats on purified synthetic diets. The salts were ground to a fine powder, mixed thoroughly and kept in a stock bottle.

Vitamine B. The water soluble vitamine B was supplied in the form of a yeast extract. Fresh, starch-free yeast (Fleishmann's compressed) was spread in thin layers and dried. When thoroughly dried it was extracted twice with ether. This ether extract was discarded. The yeast was then extracted several times with alcohol until the final extract was as colorless as pure alcohol. These extracts were combined. The alcohol was evaporated, water added, and the extract filtered with the aid of a suction filter. The volume was made up to a definite amount so that each cubic centimeter of filtrate represented a certain number of grams of fresh yeast. Usually 1 cc. represented 4 grams of fresh yeast. This extract was kept in small stoppered flasks. It was sterilized at 10 pounds pressure for ten minutes. These flasks were put away in an ice chest to be used as needed. Portions of the same extract were used for many tests. Each lot of yeast extract was tested out upon polyneuritic pigeons and found potent. However, we feel sure that this substance may constitute a variable factor in animal feeding experiments. For this reason enough extract was made at one time to carry through several tests.

Butter-fat. Butter-fat was used as a source of fat, and as a source of the vitamine A. We had found in previous work that a good quality of butter (ordinary salted butter) may be almost entirely lacking in the fat soluble vitamine during certain months of the year. We found fresh unsalted butter to be better the year round. At seasons when it was difficult to secure growth in the young rats on the adequate synthetic diet, a portion of cod liver oil was added in place of all butter. The butter oil was filtered off from the casein and water in the butter. The pure butter oil was packed away in covered jars in an ice chest to be used as needed.

Corn starch. Kingsford's corn starch was used in all diets. It was used directly from the packages. In some previous work we autoclaved quantities of this in large wide-mouthed pans at 20 pounds pressure for from two to three hours and fed this instead of the raw starch. But there appeared to be no difference in sets of animals fed the raw or the autoclaved carbohydrate.

Roughage. In making up the diets agar-agar was completely dissolved in boiling water. The other ingredients were added slowly so as to make a uniformly smooth, and a moderately dry paste. Enough of the mixture was made each time to last for two days' feeding.

Crisco. The fat was melted in a tall beaker or jar, and it was kept melted for a period of at least twelve hours while air was bubbling slowly through it. It has been shown that aeration and especially oxygenation rapidly destroys the vitamine A factor (4). For this reason this added precaution was taken in preparing the A-free fats. The fat was stored in closed tins, put away in an ice chest and used throughout the tests.

Extracts of the body fluids. Urine in 1000 cc. lots was evaporated to dryness on a water bath, the residue extracted with ether and alcohol, the extracts combined and the ether-alcohol evaporated. The total residue was thoroughly mixed with 200 grams of the A-free diet. In some cases the following procedure was used. One thousand cubic centimeter lots of urine were evaporated down to about 500 cc. and poured over 300 grams of Kingsford's starch, and this mass extracted with ether and alcohol in Soxhlet apparatus.

The saliva, bile, gastric and pancreatic juices were extracted in 1000 cc. lots by the above method, and mixed with the diets in similar proportions.

Diets of the individuals yielding the body fluids. During the periods of high A content of the diet (man) the following foods were eaten daily, in addition to other articles not rich in A: 2 to 4 eggs, 1 quart whole milk, 1 to 2 oranges, spinach and lettuce, liberal amounts of butter, three tablespoonsful of cod liver oil (Parke, Davis & Co.). The collection of urine and saliva was started twenty-four hours or more after beginning the régime.

The control diet for man included one pint of milk, one egg, one orange and some lettuce each day.

Urine of man was collected during two periods (five days and fifteen days) of absolute fasting. During these fasts water was taken as desired. The collections were started twenty-four hours after the last meal.

Copious flow of saliva was secured by chewing paraffin. Before collecting the saliva the teeth were scrubbed and the mouth thoroughly washed so as to remove possible traces of the A vitamine.

Human gastric juice. The appetite and continuous secretions were collected for me by Doctor Carlson. He had at that time in the laboratory Miss H., a child of twelve years, under his care. The child had had a stenosis of the esophagus and a gastric fistula for many years. The secretions received from this source were dried upon starch and extracted in a Soxhlet as were other body fluids.

Human pancreatic juice. This secretion was secured by Dr. A. B. Luckhardt, Fred Stangl and Dr. F. C. Koch, about nine months before the

tests were made. It was collected from a patient who had a temporary pancreatic fistula. After certain observations and analyses (5) were made upon portions of the various samples collected, the remainder was stored away in an ice chest. Each sample had been covered with a layer of toluol, and the container tightly stoppered. However, when the material was used for these tests, a wide flocculent layer was present at the surface of each fluid. This flocculent layer appeared several days after collection of the pancreatic juice. In some cases this was present also at the bottom. The toluol and a large part of the upper flocculent layer were skimmed off. Liter quantities were measured out and evaporated to half their volume. This was dried on starch and an extract made as in the other body fluids.

Dog urine. One large, healthy dog kept in a metabolism cage was fed a diet containing an excess of vitamine A. For another period the dog was fed only the ordinary diet, stock, probably low in A. Urine was collected under the varying conditions. During the period when the dog was being fed the excess vitamine A its daily diet included besides the ordinary laboratory diet for dogs, the following: 1 pint of whole milk, 2 eggs, 6 tablespoons of cod liver oil. As a rule the dog would voluntarily drink all the milk during the day. On a few occasions it was necessary to give the milk by stomach tube. The eggs were cooked, as we found it impossible to make the dog eat them raw. Three tablespoons of cod liver oil were given each morning and three were given each night. The dog took the oil from the spoon with no difficulty. All of the high vitamine A substances were fed to the dog outside of his cage in order that the urine might not be contaminated directly by this food. The urine was emptied from the collecting bottle at frequent intervals during the day. The cage was kept free from feces. Urine contaminated with feces was discarded.

Dog's gastric juice. I am indebted to Dr. T. E. Boyd for the supply of gastric juice from two Pavlov pouch dogs. Some of the samples were collected at a time when the dogs were being fed an excess of the vitamine A factor. Some samples were collected when the dogs were on the ordinary laboratory diet. These samples were not filtered. The mucus was not separated from the fluid. They were treated in the same manner as the other secretions.

RESULTS. As previously mentioned, one sometimes finds whole litters, more rarely individuals, that show no gain in weight, perhaps only an increase of 1 or 2 grams, or even a very slight loss in weight when fed for a long period (two to three weeks) upon a theoretically adequate diet, when other rats of practically the same age are gaining upon exactly the same diet.

We wish to state again that only such individuals as did show a fair gain within a reasonable time while upon the high vitamine A diet (or adequate synthetic diet) were selected for further tests. Some individuals upon

a deficient diet show a loss in weight while their general appearance seems good. Others show externally an appearance of failing nutrition even with a weight maintained at about the same level. There often comes a distinct change in disposition. Appetite is not always influenced. In many there is a gradually increasing loss in the desire to take food, but some have been observed even up to the last days before death with an appetite about as keen as that of a stock rat. In drawing a conclusion as to the presence or absence of vitamine A in any diet many considerations must be taken into account. For the final, special tests reported here, there was a distinct gain, as registered by weight of each rat, when continued for a reasonable period upon a high vitamine A diet, and also a distinct loss, as registered by weight when later each animal was placed upon a vitamine A-free diet. Curves *I* and *II* of figure 1 show that when the diet of man is very rich in vitamine A this vitamine passes into the urine in quantities sufficiently large to be detected by animal feeding experiments. The quantity appears to be less in the samples dried on starch and extracted with the Soxhlet. It was believed that extraction in a Soxhlet might give a higher value because of the more thorough extraction. It may be that a greater amount of the vitamine was destroyed in the drying of the urine upon the starch. Exposure to the air has been shown to be destructive (4). Individual rats refusing to eat the urine diet were dropped from the experiment. Most of the animals upon this diet left a very little of a 10-gram daily portion. When there is only a moderate amount of vitamine A in the diet (an amount held by some to be essential for the proper maintenance of health), there appears to be no demonstrable quantity of vitamine present in the urine. The individual curves show that one rat in this series maintained itself at practically stationary weight. All the others show some loss. One must note from the composite curve, curve *II*, figure 1, that the loss in weight is not as rapid or as great as that in rats of similar age being fed at the same time upon a purified vitamine A free diet. If one might judge from the rate or degree of loss, urine excreted by individuals on a moderate vitamine A diet appears to contain some of the A vitamine.

Curve *I*, figure 3, seems to show that saliva from persons on a high vitamine diet is free or nearly free from vitamine A. One of the persons yielded a very watery saliva as compared to the saliva of the second individual. The rats on the extract diet of the watery saliva lost weight more rapidly than those on the extract from the thick saliva. As these were rats from the same litter, the experiment may mean a trace of vitamine A in mucous saliva.

The quantity of human gastric juice available for the tests was not large. Curve *II*, figure 3, shows the composite curve of two animals which were run for eight days on the continuous secretion diet. It is evident that this secretion was distinctly negative. The appetite secretion seemed less

dilute and it is seen in curve *III*, figure 3, to produce a less negative curve than the continuous secretion. The child from whom the gastric secretions were collected was, at the time of the collection of the secretion, upon a moderately high vitamine diet. Even so she was pale and showed a certain degree of malnutrition.

The samples of human pancreatic juice were negative, curve *IV*, figure 3. The patient from whom the juice was collected was not on a rather high vitamine A diet. The juice may have undergone changes during the period of storage—however, no vitamine A effect was produced. If any vitamine

Fig. 1. Showing growth of rats.

Curve *I* (composite of 8). High vitamine A. Human urine extract.
Curve *II* (composite of 5). High vitamine A. Soxhlet extract of human urine.
Curve *III* (composite of 7). Controls on A-free diet.
Curve *IV* (composite of 6). Ordinary diet. Human urine extract.
———— Adequate diet; ---------- vitamine A-free diet; xxxxxxxx special urine extract diets.

Fig. 2. Showing growth of young rats on starvation urine extract diets, and controls.

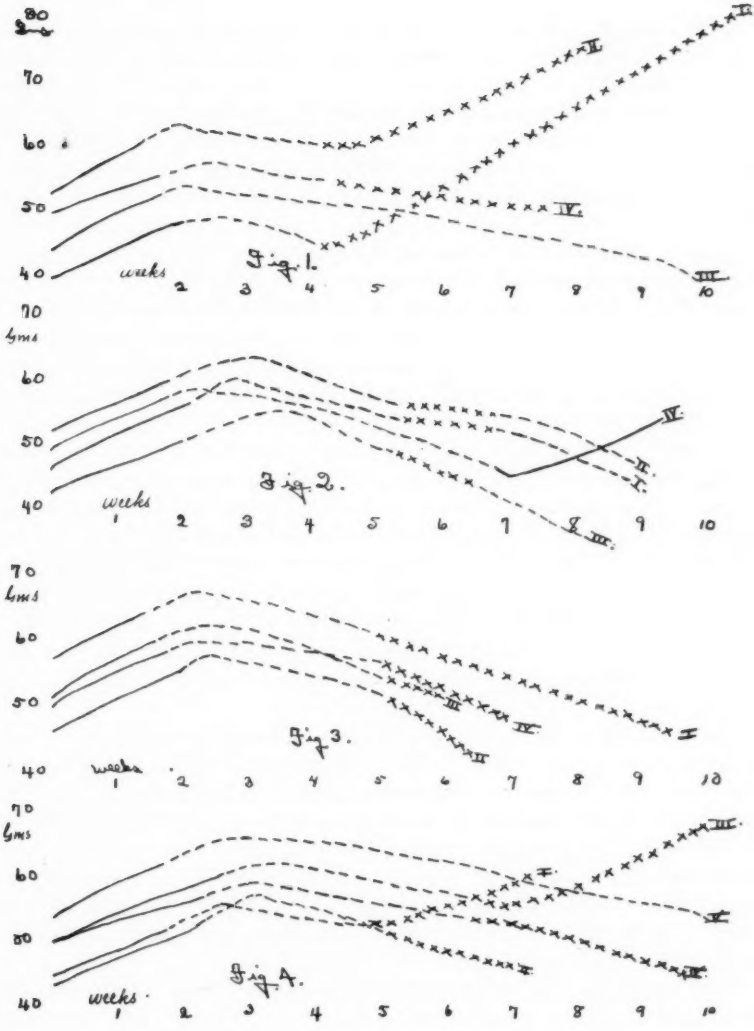
Curve *I* (composite of 4). Extract of 1st five days' starvation urine (human).
Curve *II* (composite of 5). Extract of 2nd five days' starvation urine (human).
Curve *III* (composite of 5). Extract of 3rd five days' starvation urine (human).
Curve *IV* (composite of 5). Controls kept on A-free diet during experimental period of other 3 sets—then put on an adequate diet and recovered the loss in weight. The 3 sets of rats on starvation urine extract (human) were put upon the known A-free diet and showed a more rapid loss in weight.
———— Adequate diet; ---------- vitamine A-free diet; xxxxxxxx special urine extract diet.

Fig. 3. Showing growth of young rats on various body fluids from man.

Curve *I* (composite of 6). Human saliva; high A diet.
Curve *II* (composite of 2). Human gastric juice. Continuous secretion.
Curve *III* (composite of 2). Human gastric juice. Appetite secretion.
Curve *IV* (composite of 3). Human pancreatic juice extract diet.
———— Adequate diet; ---------- vitamine A-free diet; xxxxxxxx special body fluid extract diet.

Fig. 4. Growth of rats when on extract from dog's gastric juice and dog's urine.

Curve *I* (composite of 5). Extract of dog's gastric juice from high vitamine A diet.
Curve *II* (composite of 3). Extract of dog's gastric juice from ordinary laboratory diet.
Curve *III* (composite of 10). Extract of dog's urine from high vitamine A diet period.
Curve *IV* (composite of 10). Extract of dog's urine from ordinary laboratory diet.
Curve *V* (composite of 8). Rats continued on known vitamine A-free diet.
———— Adequate diet; ---------- known vitamine A-free diet; xxxxxxxx special extract diet.


80
70
60
50
40
weeks
1 2 3 4 5 6 7 8 9 10
I.
II.
III.
IV.
Fig. 1.
70
60
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I.
II.
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Fig. 2.
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1 2 3 4 5 6 7 8 9 10
I.
II.
III.
IV.
Fig. 3.
70
60
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40
weeks
1 2 3 4 5 6 7 8 9 10
I.
II.
III.
IV.
Fig. 4.

A had been present, it may have been adsorbed in the flocculent portion. Most of this was removed from the samples as was mentioned under "method."

It is known that vitamine A is stored in many fats (6), (7), and also that it is found in some other body tissues, heart, kidneys, lungs, muscle, glands and nervous tissue. During the periods of total starvation it seemed possible that some vitamine may be set free at the same time the fat and other tissues were being used up. In 1919 Hopkins (10) observed that young *nursed by females* receiving a diet *deficient in vitamine A* grew to about one-half their normal size and showed many signs of undernourishment. It was observed by Decairne (8), during the siege of Paris, that even during fasting or partial fasting vigorous young mothers were able to keep up such a flow of milk as was sufficient to *induce some growth* in their infants. For growth, all of the fundamental factors including the vitamine A, must have been present in the breast milk. Kennedy and Dutcher state that the cow may secrete vitamines A and B into her milk for some time after the vitamine supply in the food has been diminished (9). During the fast of fifteen days (conducted as a part of the experiment reported here) about 9 liters of urine were collected. That collected during the first five days was extracted and tested separately on one series of rats. That collected on each of the succeeding five-day periods was extracted and tested separately on other series of rats. Curves *I*, *II* and *III* of figure 2 show the urine during the entire period to be lacking in A. The result from the second starvation period of five days was practically the same as that shown in curve *II*, figure 2. But from the same figure it is observed that at the end of the special starvation urine test, the animals showed a *more rapid loss* in weight when put upon the known vitamine A-free diet.

The quantity of urine and the other fluid extracts on the high vitamine A diets, given to each animal daily, seems large but the growth of the rats was moderate. It may be that during a prolonged fast only a very small amount of stored vitamine A is set free daily and that this is used up in the metabolism processes so none is given off in the urine. Considerable amounts of vitamine A may be set free during a fast period and some may escape in the urine and yet not be detected because of the small amount present, or because much of it may have undergone destruction in the process of preparing the extract.

Figure 4 shows composite curves from tests made upon the urine of one large laboratory dog. With an excess of vitamine A in the diet, a sufficient amount passed into the urine to produce a distinct increase in weight of rats. The urine extract from the dog on the ordinary laboratory diet (meat, bread and table scraps) did not prevent a definite loss in weight. Curves *I* and *II* of figure 4 show dog's gastric juice to contain the vitamine A only when there is an excess of vitamine in the diet. None of the animals upon the gastric juice extract diets refused to eat their food.

SUMMARY

1. On diets high in vitamine A, the urine (man, dog) contains a demonstrable quantity of this vitamine. On ordinary diets and in prolonged starvation the urine is nearly, if not completely, free from vitamine A.

2. Pure gastric juice (Pavlov pouch) dogs on high vitamine A diets contain vitamine A, but on ordinary diet the gastric juice of man and dog appears to be free from this vitamine.

3. Demonstrable quantities of vitamine A are not present in human saliva (high and low A diet), or in the old pancreatic juice (high diet).

4. It seems probable that the kidneys and the digestive glands have a "threshold" limit for vitamine A retention in the body. When the body is flooded with this vitamine by an excess in the diet, the absorption is faster than the storage and destruction, there follows a rise of the vitamine concentration in the blood, and consequent vitaminuria, and overflow into some of the digestive secretions. Since a considerable part of the vitamine A in milk seems to be in solution outside the milk fat, the threshold limit for vitamine A overflow in the mammary gland is either relatively low, or else we are dealing with a true vitamine A secretory process in this gland.

I am greatly indebted to Dr. A. J. Carlson, to Dr. A. B. Luckhardt, to Dr. F. C. Koch, for advice and suggestions on many parts of the problem. I am very grateful to Dr. T. E. Boyd for his generosity in supplying me with samples of gastric juice from his experimental dogs.

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EFFECT OF INTRAVENOUS SALINE SOLUTION ON THE LEUCOCYTE COUNT

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In treating dementia praecox patients with intravenous infusions of physiologica' normal salt solution, the writers have investigated the effects of such infusions upon the leucocyte count of the blood. For comparative purposes they studied three groups of cases, consisting of dementia praecox patients, normal individuals, and febrile patients with high initial leucocyte counts.

Six dementia praecox patients were each given 1 liter of 0.9 solution of sodium chloride intravenously at approximately blood temperature. The average leucocyte count of these six patients before the infusions was 8,133. The average count at the end of six hours was 11,327, showing an increase of 39 per cent over the original count. The average counts at different intervals were as follows:

Count before the infusion	8,133
After three hours	10,666
After six hours	11,327
After nine hours	9,180
After twenty-four hours	6,708

TABLE 1

	COUNT BEFORE INFUSION	AFTER 3 HOURS	AFTER 6 HOURS	AFTER 9 HOURS	AFTER 24 HOURS
Dementia praecox no. I	7,300	13,200	12,233	8,800	6,600
Dementia praecox no. II	8,400	12,300	13,630	11,722	7,350
Dementia praecox no. III	7,700	10,033	11,750	8,150	5,600
Dementia praecox no. IV	6,900	11,500	12,300	10,210	9,400
Dementia praecox no. V	8,600	9,300	10,700	9,550	5,850
Dementia praecox no. VI	9,900	7,666	7,350	6,650	5,450
Average for six patients	8,133	10,666	11,327	9,180	6,708

The counts for the individual patients are given in table 1. It will be seen that while the first five patients show a marked increase in leucocytes, the sixth patient shows a slight decrease. If computations are made only for the five patients showing an increase, we find an average count at the end of six hours of 12,123, this representing a 56 per cent increase in leucocytes, instead of 39 per cent increase for the total group.

The second group of six subjects were normal individuals—members of the faculty and student body of the Medical School of the University of Colorado. The average leucocyte count for this group before the infusions was 5,433. At the end of six hours there was an increase of 66 per cent, the average count being 9,050. The average counts at intervals were as follows:

Count before the infusion	5,433
After three hours	7,450
After six hours	9,050
After nine hours	8,522
After twenty-four hours	6,767

TABLE 2

	COUNT BEFORE INFUSION	AFTER 3 HOURS	AFTER 6 HOURS	AFTER 9 HOURS	AFTER 24 HOURS
Normal no. I	7,500	9,100	11,300	10,100	8,200
Normal no. II	4,800	6,400	8,100	6,600	6,500
Normal no. III	5,500	6,100	8,600	8,300	4,100
Normal no. IV	5,400	7,600	9,400	9,030	8,100
Normal no. V	4,800	5,700	8,200	8,700	5,650
Normal no. VI	4,600	9,800	8,700	8,400	8,050
Average for six cases	5,433	7,450	9,050	8,522	6,767

Counts for the individual subjects are recorded in table 2.

Further investigations were made with pneumonia patients who were to be treated with saline infusions, the counts being made relative to the first infusion received. The average leucocyte count before the infusion was 25,184. At the end of the six hours the leucocytes had increased only 5 per cent, the average count being 26,600. The average counts at intervals were as follows:

Count before the infusion	25,184
After three hours	24,433
After six hours	26,600
After nine hours	21,050
After twenty-four hours	18,950

TABLE 3

	COUNT BEFORE INFUSION	AFTER 3 HOURS	AFTER 6 HOURS	AFTER 9 HOURS	AFTER 24 HOURS
Pneumonia no. I	24,900	24,400	27,500	22,600	19,300
Pneumonia no. II	21,000	21,400	29,500	20,700	23,200
Pneumonia no. III	34,000	34,200	37,500	27,200	25,800
Pneumonia no. IV	24,700	21,800	23,100	20,800	16,200
Pneumonia no. V	27,000	26,200	22,200	18,600	14,000
Pneumonia no. VI	20,400	18,600	19,800	16,400	15,200
Average for six cases	25,333	24,433	26,600	21,050	18,950

The counts for the individual cases in this series are shown in table 3.

These investigations seem to show that intravenous infusions of normal salt solution cause a marked increase in the leucocytes of the blood if the number of leucocytes is normal when the infusion is given. The leucocytosis was at its highest at the end of six hours in a series of counts made at three-hour intervals.

No material increase in leucocytes ensues if there is a high leucocytosis before the infusion.

THE POSSIBLE HEREDITARY FACTORS IN EXPERIMENTAL PRODUCTION OF EDEMA AND XEROPHTHALMIA

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This investigation was stimulated by the following hypothesis, suggested by Doctor Carlson. First, on a diet too low in protein not all animals show edema; second, on a diet deficient in fat soluble A vitamin not all animals develop xerophthalmia. This resistance to edema and xerophthalmia may be a hereditary factor.

There are some additional results and conclusions reported here, not directly bearing upon the problem of heredity in these deficiency diseases. Nevertheless, the work was stimulated by the above original hypothesis and is all reported here.

Literature. Kohman (1) was unable to explain why some of the rats on the low-protein-carrot diet develop edema and others do not. She questions why the tissues of one rat undergo changes which cause them to retain fluid to an excessive degree and those of another rat do not undergo the same changes, when both rats are from the same litter, have the same weight and are receiving equal amounts of the same food. Walker (2) observed xerophthalmia in rats on a vitamin A free diet in only five out of sixty-four rats in one case and found six out of thirty-eight rats in another case on a diet lower in protein. This being unlike the figures of Emmett (120 out of 122) or Osborne and Mendel (69 out of 136) he points to the possibility, suggested by Carlson, that there may be a hereditary predisposition or idiosynerasy in the development of xerophthalmia. Hopkins (3) observed that when two sets of rats from entirely different stock were both put on a diet containing fat aerated for four hours at 120°, one set developed xerophthalmia and the second set showed no symptoms. He states that "the influence of stock and perhaps of nutrition preceding the experiment may affect the incidence of keratomalacia." Funk and Dubin (4) noted that among thirty rats two developed ophthalmia on a diet containing the usual amount of vitamin A. One of them was improved by the addition of yeast to the diet. The other was improved without medication. Bulley (5) feels that the occurrence of xerophthalmia is due to an infection. She states that her animals

were free from xerophthalmia, because they were kept under the cleanest and healthiest conditions, care being taken to treat the sore eyes with boracic acid.

Experimental xerophthalmia. In order to develop xerophthalmia in rats the so-called synthetic diet was used. The ingredients and the percentage used are as follows:

	per cent
Meat residue (Parke, Davis and Co.)	20
Corn starch (Kingsford's)	50
Salt mixture (McCollum's no. 185)	5
Yeast extract (Fleischmann's)	5
Butter (filtered)	20
Agar-agar	3

Rats, weighing from 50 to 60 grams, from selected stock raised in our own laboratory, were used throughout this experiment. Care was taken to keep the rats in as sterile condition as possible.

In no cases did xerophthalmia occur in rats when vitamin A was removed from the diet. In the first place, all the rats did not respond to the diet, when vitamin A was present, cod liver oil being substituted for part of the butter in some diets. Therefore substitution of aerated Crisco (cotton seed oil) as an "A-free" fat was not possible. In the second place, where response to the synthetic diet was made, loss in weight, rough hair coat and atonicity of the muscles occurred, but no eye symptoms. An unbalanced condition was present in a large number of the rats on an A-free diet. This condition was characterized by inability of the animals to walk or to hold a balanced standing position. Several of these animals tended to hold their heads to one side and showed a circus movement when walking. Thinking this condition might be related to polyneuritis, special care was taken to have more than the adequate amount of vitamin B in the diet. No change in symptoms occurred.

Previous work in this laboratory points to the fact that "meat residue" may not be an adequate protein in the diet of the rat. Therefore, casein was prepared from skimmed milk according to the process described by Kohman (1). Casein was substituted for "meat residue" in the diet, but similar results were obtained. The casein was then prepared from a commercial product by dissolving and reprecipitating, following out the purification as with the skimmed milk product. Again, the results were similar to those with "meat residue" protein. The protein in the diet was changed the fourth time using "Harris vitamin A and B free casein," only to result in symptoms similar to the above.

Dissatisfied with results, so contrary to reports from other laboratories, another less purified diet was used in attempt to see if it was possible to produce xerophthalmia with any degree of regularity. The following is the diet used by McCollum (6) for producing xerophthalmia:

	grams
Steel cut oats	40
Gelatin (Bacto)	10
Dextrin	46.3
Salt mixture (no. 185)	3.7

Two different sets of rats, weighing from 50 to 60 grams, were placed on this diet. One set was given 2 per cent of butter in place of an equal amount of dextrin for four weeks during which time there was a normal gain in weight. Then butter was removed from the diet. The rats continued to gain slowly for three months. Xerophthalmia has developed in no cases at the present time, although the rats are not normal in size or vigor.

The second set of rats were placed on a vitamin A free diet immediately. They gained slowly, 2 to 5 grams per week, for three or four weeks and then showed a loss of weight followed by definite eye symptoms. One litter showed 83 per cent of xerophthalmia in nine weeks, another litter 43 per cent in ten weeks. A third litter on the the same diet showed no eye symptoms, but with loss of weight came loss of hair coat in patches. Eighty per cent of this litter showed these symptoms in from three to four weeks when simultaneously they began to lose in weight.

Experimental edema. Four litters, each rat weighing between 35 and 45 grams, were started on the following low-protein-carrot diet used by Kohman (1).

	grams
Carrots	4500
Starch	228
Butter	120
Salt II	19.2
Salt III	16.4
Yeast extract	50.0

The original litters on this diet are numbered in the following table, I, II, III, IV. The cases of edema developed, the time in which edema appeared and the percentage of rats per litter which developed edema are recorded in the table.

Attempt was made in every case to restore to normal the edematous rats by giving them a regular stock diet. If successful recovery occurred these rats were mated, in most cases brother and sister, thus eliminating as far as possible, variable hereditary factors. The young of these matings are recorded as F_1. After producing edema in F_1, those rats were brought back to normal and mated. The offspring from these matings are recorded as F_2. In two cases there is a record of stock rats of normal parentage run on the same diet at the same time with rats of edematous parents.

TABLE 1

Showing the average time in which rate in each litter developed edema and the number and percentage of rats per litter which developed edema

LITTER	AVERAGE TIME IN WHICH EDEMA DEVELOPED	NUMBER OF CASES PER LITTER	PERCENTAGE OF CASES PER LITTER
	days		
I (normal parents)	32.5	6.0	83.3
F_1 (edema parents)	35.65	3.0	60.0
	41.0	2.0	25.0
	49.7	8.0	88.0
	45.2	4.0	50.0
	39.0	5.0	66.6
	31.0	2.0	50.0
F_2 (edema parents and grandparents)	41.7	4.0	66.6
	24.0	1.0	33.3
	40.0	5.0	71.3
	41.6	6.0	66.6
	19.0	6.0	75.0
	32.0	4.0	50.0
	21.6	3.0	75.0
	29.2	4.0	57.1
	43.0	3.0	42.7
	48.0	2.0	22.2
II (normal parents)	41.0	4.0	66.6
F_1 (edema parents)	60.5	4.0	66.6
	26.0	3.0	50.0
F_2 (edema parents and grandparents)	73.0	1.0	50.0
	27.0	1.0	16.6
III (normal parents)	59.0	4.0	42.6
F_1 (edema parents)	60.5	5.0	83.3
	52.1	7.0	63.0
	45.3	9.0	70.0
	52.0	7.0	58.3
F_2 (edema parents and grandparents)	35.5	2.0	40.0
	22.0	5.0	83.3
	73.0	1.0	50.0
	27.0	1.0	16.6
	15.0	2.0	28.5
	57.0	4.0	50.0
	42.0	7.0	100.0
	70.8	5.0	55.0

TABLE 1—*Concluded*

LITTER	AVERAGE TIME IN WHICH EDEMA DEVELOPED	NUMBER OF CASES PER LITTER	PERCENTAGE OF CASES PER LITTER
	days		
Stock (normal parents)	44.0	7.0	87.5
	60.8	5.0	71.5
IV (normal parents)	29.0	1.0	14.6
F_1 (edema parents)	41.0	2.0	25.0
F_2 (edema parents and grandparents)	41.7	4.0	66.6

A recent series of five papers has been published by Slonaker (7) on the effect of a low protein diet on the life cycle and inheritance of the rat. The average span of life of the rat on a normal diet is reported by Slonacker as thirty-four months. In our work the rats which recovered from the low protein diet reached a maximum span of life of nineteen months decreasing in others to eleven months. With a decrease in the span of life there was a relative decrease in the number of young per female.

Due to interest in the production of edema experimentally and to the fact that in a certain part of Germany this disease was called "Rubenkrankheit" (8), turnips were used in place of carrots in the edema-producing diet. Due to a difference in the caloric value of carrots and turnips the diet was changed as follows:

	grams
Turnips	4500
Starch	280
Butter	240
Salt II	19.2
Salt III	16.4
Yeast extract	50.0

The following are the results of two litters which were placed on the low-protein-turnip diet.

LITTER	AVERAGE TIME IN WHICH EDEMA DEVELOPED	NUMBER OF CASES PER LITTER	PERCENTAGE OF CASES PER LITTER
I	42	3	36.65
II	40	4	57.1

The following observations were made on a litter of six rats placed on a diet, 3 per cent of which was protein, "meat residue" being used. Five rats of the six developed an unbalanced condition in an average time of sixty-two days, dying within two or three days after this condition was

observed. No edema was observed in these rats. The unbalanced condition was the same in its characteristics as the symptoms observed on the synthetic diet used to develop xerophthalmia.

Discussion. In the attempt to produce xerophthalmia, experimentally the purification of the protein is important. "Harris casein" is prepared according to a method approved by Osborne and Mendel. Osborne and Mendel (9) have developed a more elaborate technique of purification of the ingredients of vitamin A free diets. The casein is boiled three times with absolute alcohol under a reflux condenser and filtered off by suction. Funk (10) recently reports on the presence of a fat soluble substance in purified casein. He is adopting a new method of casein purification consisting of repeated boiling out with alcohol.

If loss of weight, failure to grow, rough hair coat and inanition are used as evidence of the absence of vitamin A, we would be able to report on the response of rats to the absence of vitamin A in more cases than if xerophthalmia is the only criterion used. However, we cannot account for the fact that not all rats responded to the presence of vitamin A in the so-called adequate, synthetic diet.

Eye symptoms did occur in our laboratory in rats on a less purified diet. The presence of oats in this diet brings in variable and unknown factors. We cannot account for the fact that xerophthalmia occurred in two litters on this diet while a third litter put on the diet at the same time, though showing an equal loss of weight, showed no eye symptoms, but a change in hair coat. This unexpected result seems to point again to the hereditary factors in the experimental production of deficiency diseases. But our data on the production of edema give no adequate proof of a hereditary factor. There seems to be no greater percentage of rats from edematous parents or grandparents developing edema than rats from normal parents developing edema. Also, the time in which the edema develops seems to show no increase or decrease in rats with edema parents or grandparents.

We failed to produce edema on a diet 3 per cent of which was adequate protein, "meat residue." But the rats on the "meat residue" diet as well as on the diets in which the protein was purified casein (up to 20 per cent of the meal) usually developed the "unbalanced" condition previously noted, and did not do as well as our rats on the stock diet. The same difficulty or impossibility of securing normal growth and nutrition in rats on highly purified but theoretically adequate diets has been experienced by other workers in this laboratory (Kohman, Cooper). The cause of this is at present unknown. Monotony in the diet may be a factor. There may be injury to the proteins in the process of purification or the various purification processess may develop traces of toxic substances that produce injuries on continued feeding.

The effect of diet on successive generations, especially as to span of life and reproduction, is an important factor in experimental research with animals. Davis and Outhouse (11) show the effect of a ration low in vitamin A on the span of life and reproductive activity of the rat. The presence of this vitamin is no doubt of great importance for the rearing of normal animals.

CONCLUSIONS

1. On the synthetic vitamin A free diets xerophthalmia was not produced in our rats. On a more natural and less purified diet xerophthalmia occurred in two out of three litters.

2. The present experiments reveal no definite factor of heredity in the development of edema in rats on a low protein diet.

3. Low protein diets decrease the span of life and the number of offspring.

I wish to express to Dr. A. J. Carlson and to Miss Ethel F. Cooper my appreciation of their interest and helpful suggestions.

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THE EFFECTS OF TEMPORARY ANEMIA ON THE TONE OF THE BLOOD VESSELS

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The work here reported was undertaken with the view of determining whether the post-anemic spasms of the blood vessels observed by Carlson and Stoll in the kidneys are peculiar to the renal blood vessels, or are present in the blood vessels in all the organs of the body. This point must be settled before we can proceed with an analysis of the mechanism of the spasms in any particular organ. The phenomena of post-anemic vascular spasm are also of practical interest in connection with resuscitation, various accidental asphyxias and acute hemorrhages, and in organ transplantation by vascular anastomosis. It is also clear that if post-anemic vascular spasm is a general reaction of blood vessels, this is a factor to be considered in most experiments with organ perfusion.

I. GENERAL METHODS. Dogs were used in these experiments. Almost all the dogs were given veronal by stomach tube about an hour before the experiment. Two dogs were anesthetized with ether only. The animals were kept warm throughout the experiment.

The recorder in all experiments was a water manometer of 5 mm. bore with a glass float and writing point. . It was best adapted for these prolonged experiments where absolute absence of leak was essential. The different plethysmographs will be described below.

The clamping of either artery or of veins was done with the least mechanical disturbance and with rubber covered forceps.

II. KIDNEY. *A. Special methods.* The left kidney was used in all but one experiment. Approach was made intraperitoneally. The kidney was freed with great care leaving only the ureter and renal blood vessels and nerves intact. In some the renal nerves were dissected away, ligated and cut. In the rest the artery was merely isolated from vein and nerves for clamping.

The balloon plethysmograph described by Carlson and Stoll[2] was exclusively used with the kidney. It was less cumbersome than Roy's and more easily kept leakless than Jackson's. Urine flow was registered by the drop method, the cannula being connected with the ureter near

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[2] Stoll and Carlson, This Journal, 1923, lvii, 153.

the bladder. The initial flow of urine after the release of the artery was taken at the first perceptible increase in the size of the drop. Arterial blood pressure was recorded from the carotid artery.

In experiments where the vein was clamped, either the recorder was allowed to remain connected with the plethysmograph during the experiment or was clamped off while the venous clamp was on.

B. Results. 1. Occlusion of renal artery. Nineteen experiments on 8 dogs were performed. No changes in arterial blood pressure corresponding to the changes in kidney volume were noted. Often the arterial blood pressure was uniform throughout. Sometimes there was a slight (1 to 3 mm. Hg) and temporary ($\frac{1}{4}$ to 1 minute) increase on clamping the renal artery and a similar fall at release of the blood vessel, apparently due to the increase and decrease respectively of the resistance to the general circulation occasioned by the clamping.

a. Volume changes. Essentially similar results were obtained as those by Carlson and Stoll. Immediately after clamping, the kidney volume decreases at first suddenly then more slowly reaching its lowest level in about half a minute, maintaining this level until the end of occlusion (figs. 1 and 6). After release of the artery the volume record rises in half a minute, to a level still below that of the control period, more gradually falls again, slightly in some, but almost to the occlusion level in a greater number, and then still more gradually increases to control level. This height is reached from 3 to 45 minutes after release (table 1). This secondary increase in volume may not reach the control level. In such cases the end of the "spasm" was taken as at the peak of such rise. The secondary increase was followed by gradual fall.

Table 1 shows a great tendency for the duration of the vascular "spasm" to vary in the same direction as the length of the occlusion period.

In one experiment where the aorta was clamped, instead of the renal artery, volume curve similar to clamping renal artery was obtained. In this experiment the aorta was previously ligated peripheral to the origin of the renal artery.

No changes in the size of the renal artery at or around the point of clamping were observed after the release corresponding to the volume changes in the kidney.

b. Anuria following clamping of renal artery. Six experiments were performed on the same dog. Immediately on clamping the renal artery the urine flow stops. There was neither increase nor decrease in the size of the drop already forming before the clamping until 4 to 5 minutes after the release. Table 2 shows the approximately similar duration of the vascular "spasm" and the anuria following release of the clamped renal artery in these experiments. After the temporary anuria the rate of urine flow was either faster or slower than it was during the control period.

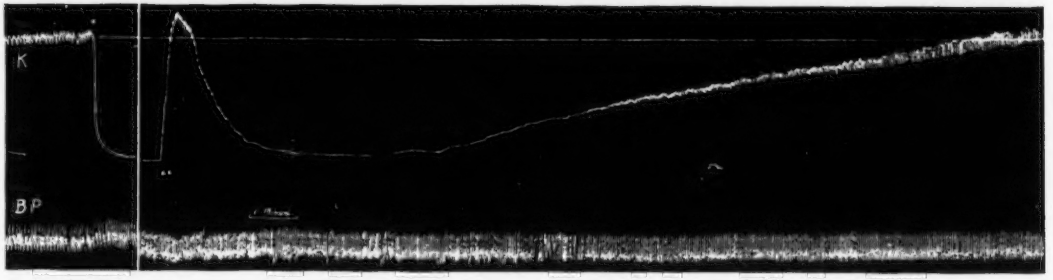

Fig. 1. Dog. Veronal anesthesia. *K*, volume of kidney (plethysmograph); *B.P.*, carotid pressure; *x*, occlusion of renal artery; *xx*, release of renal artery. Showing prolonged vascular spasm in the kidney following a 15-minute period of anemia.

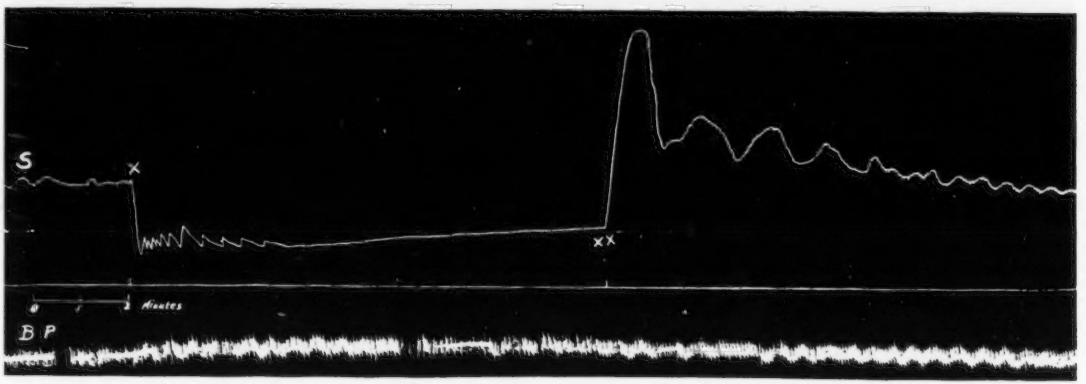

Fig. 2. Dog. Veronal anesthesia. *S*, plethysmograph volume of spleen. *B.P.*, carotid blood pressure; *x*, occlusion of splenic artery, showing periodic spasms of the spleen (vascular or extra-vascular) following 10-minute period of anemia.

2. Occlusion of renal vein. In all three experiments in which the renal vein was occluded, the kidney volume decreased, after release of the clamp, markedly below the control level, to return more gradually to that level in 2 to 10 minutes. This decrease in kidney volume below control level occurred even after release of the vein at the time when the kidney was expanding, as a result of the venous occlusion.

TABLE 1

Comparison of duration of post-anemic vascular spasm with length of occlusion of renal artery

NUMBER OF EXPERIMENTS	DURATION OF OCCLUSION	DURATION OF SPASM IN MINUTES		
		Average	Minimum	Maximum
	minutes			
1	1	3	3	3
5	5	4	3	4
6	10	9	4	20
5	15	18	4	45
2	20	17	4	30
19	20	10	3	45

TABLE 2

Comparison of duration of post-anemic vascular spasm with duration of anuria after temporary anemia of kidney of dog 9

EXPERIMENT	DURATION OF SPASM	INITIAL FLOW MINUTES AFTER RELEASE	FIRST DROP MINUTES AFTER RELEASE	RATE OF URINE FLOW AFTER RELEASE (+ = INCREASE − = DECREASE)
	minutes			
1	4.0	*	4.0	+
2	4.0	*	4.0	−
3	4.5	5	6.0	−
4	4.0	4	5.0	+
5	4.0	4	5.0	+
6	4–5	5	5.5	−

* Not observed.

3. Electric stimulation of renal nerves. This was performed both when the kidney was in the oncometer and when the kidney was out of it and totally excised. On stimulation of the peripheral end of the cut renal nerves the kidney volume fell and on stopping the stimulation the volume gradually returned to normal in 2 to 10 minutes. There was no subsequent contraction of kidney as was present after temporary anemia by clamping the renal artery. The absence of vascular spasm following stimulation of the renal nerves is probably due to the anemia being either incomplete or of too short duration.

In two experiments on excised kidney in which cannulae were connected to both artery and vein, there was marked acceleration of venous flow with some back flow in the artery.

Discussion. There are three possible causes of changes in kidney volume in our experiments: 1, vascular changes; 2, transfer of fluids from the kidney tissues to blood and vice versa; and 3, changes in the amount of urine inside the kidney either as a result of changes in resistance of ureter or reabsorption of contents of the uriniferous tubules into the circulation. Experiments in which ureteral changes in resistance to the flow of urine was eliminated by cannula being pushed into the pelvis gave the same results.

If the failure of the kidney to return to its control size, for 3 to 45 minutes after release of the artery or for 2 to 10 minutes after release of the vein was due to transfer of fluid either from the tissues or the tubules to the circulation, such transfer must take place during the occlusion. But it is quite evident that the failure was caused by a condition change at or immediately after the release of the artery or vein. The occlusion level was either horizontal in artery occlusion, or increasing in vein occlusion. Besides, the kidney volume after release of artery often increases up to, or even above, control level before it contracts for a more or less extended period. Hence it must be vascular changes that cause the continued contraction of the kidney after release of occluded artery or vein.

These vascular changes could not be the result of decreased general arterial blood pressure as there was no such change. It must therefore be an increased tone of the kidney blood vessels. Such increased vascular tone could not be due alone to asphyxial condition for in that case it should occur during the occlusion. It could be produced by 1, sudden change of pressure inside the blood vessels acting as mechanical stimulus; 2, such a mechanical stimulus together with increased irritability of the blood vessels resulting from the asphyxial condition; or 3, normal blood acting as a chemical stimulus on blood vessels rendered more irritable by the previous asphyxial condition. It is probably not due to mechanical tension stimulus alone, for if it were, the duration of spasm should not vary with the duration of anemia. That such change of tension is a factor is rendered probable by the fact that it is a condition change at the moment of release of the artery and by the further fact that no spasm was noticed when the general blood pressure was low.

The ultimate return to normal volume is presumably due to relaxation of this increased vascular tone. It is not due to gradual accumulation of urine. In experiments in which the ureteral cannula was pushed into the pelvis to eliminate possible resistance by partly occluded ureter, similar curves were obtained. Local edema coming on a few minutes after

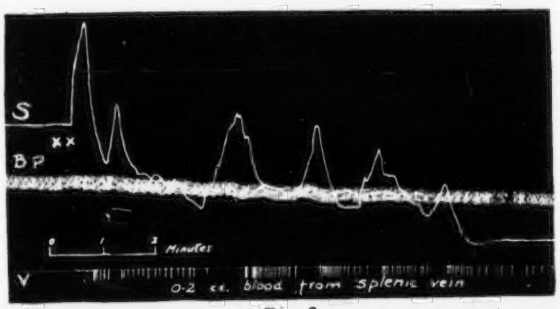

Fig 3

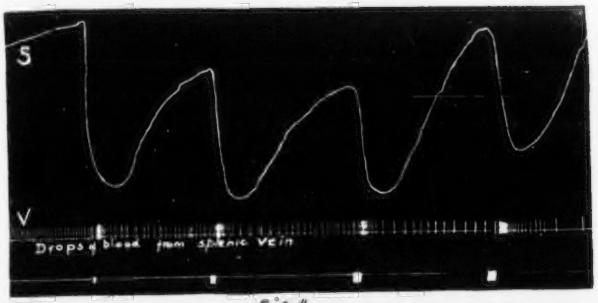

Fig 4

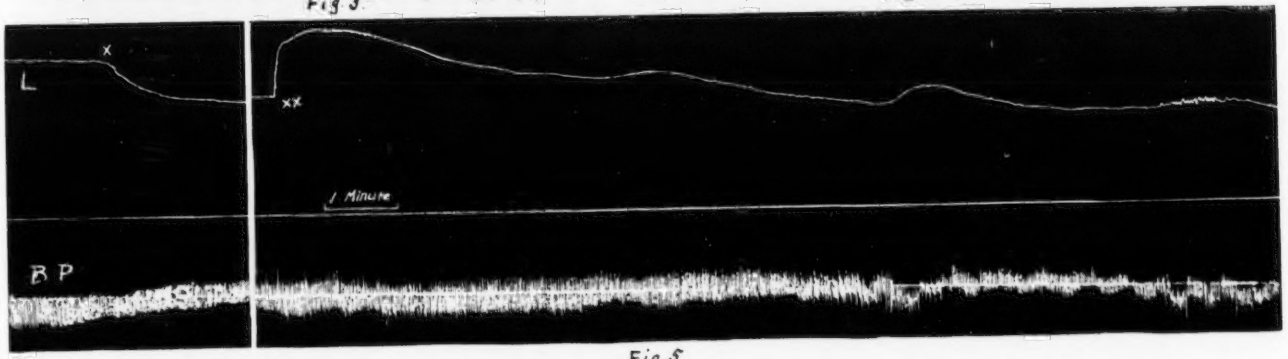

Fig 5

Fig. 3. Dog. Veronal anesthesia. *S*, plethysmograph volume of the spleen, the splenic artery being clamped; *B.P.*, carotid blood pressure; *V*, record of blood flow from the cut splenic vein; *xx*, release of splenic artery. Each signal mark = 0.2 cc. blood. Showing increased venous flow on contraction of spleen volume, and reduced flow or cessation of flow when the spleen dilates.

Fig. 4. Dog. Veronal anesthesia. *S*, plethysmograph volume of spleen; *V*, record (drops) of flow of blood from cut splenic vein; signal, tetanization of peripheral end of cut splenic nerves. Showing great augmentation of venous outflow on contraction of spleen, and a retardation of the blood flow on dilatation of spleen.

Fig. 5. Dog. Veronal anesthesia. *L*, plethysmograph volume of the leg; *B.P.*, carotid blood pressure; *x*, occlusion of femoral artery; *xx*, release of occluded artery. Showing periodic vascular spasms in the leg after a 10-minute period of anemia.

release of the occluded artery might be a factor. That this is possible is shown by the result in one experiment on the thyroid, where great accumulation of lymph was noted 15 minutes after temporary anemia. But in the case of the kidney this explanation is rendered improbable by the production of as much decrease of kidney volume on subsequent occlusions as on the first, when controls in all cases were at the same level.

Anuria after arterial clamping. These experiments confirm the findings of Carlson and Stoll that under conditions in which reflexes into the kidney could not take place anuria followed temporary occlusion and release of the renal artery. They found a great tendency for the duration of anuria to vary with the duration of occlusion. The experiments here reported show a similar tendency for the duration of the spasm to vary also with the length of occlusion. Besides, in the experiments where simultaneous record was made of urine flow and volume of kidney, the anuria lasted within half a minute as long as the spasm. A direct causal relation between anuria and vascular spasm after temporary occlusion and release of renal artery is therefore very probable.

III. SPLEEN. *A. Special methods.* The omentum is freed from the spleen. All blood vessels except the main artery and vein were ligated and cut. In some cases the splenic nerves were ligated and cut, but in most cases they were left intact, the artery or vein being dissected away from the nerves for clamping. This leaves the spleen with a small pedicle composed of nerves and the two main blood vessels. The plethysmograph was made out of a small mouthed glass bottle of 7 cm. diameter with the bottom cut off and the edge ground. A hole larger than the splenic stem was punched through a soft rubber stopper fitted to the cut end of the bottle. The stopper was then slit through from the hole to the edge. To put the spleen in the plethysmograph, the hole in the stopper was filled with vaseline, the slit was opened and the stem of the spleen was passed through it to the hole. The bottle and the stopper were fitted together. Connection with the recorder was made through the mouth of the bottle.

Arterial blood pressure was recorded from the carotid artery.

The splenic artery was clamped for 1 to 10 minutes in 9 experiments on 3 dogs, the vein for 1 to 8 minutes in 4 experiments on one dog. Blood flow from the vein after temporary arterial occlusion was determined in one experiment. In this experiment, while the spleen was in the oncometer the artery was clamped and when the spleen volume (as shown by the recorder) apparently reached its lowest limit, the vein was clamped and a 100 cc. burette containing 5 per cent sodium citrate solution was connected with the splenic end of the vein. The burette was clamped in an almost horizontal position with the citrate level slightly higher than the level of the vein. The artery was released 25 minutes after clamp

was put on. One of us observed and recorded the rise of the citrate level for every 0.2 cc. mark of the burette.

The effect of stimulation of the cut splenic nerves on the volume of the spleen and the blood flow through the organ was performed in the same manner. The nerves were also stimulated while the splenic artery was clamped, the venous outflow being recorded by drops.

Two excised spleens were perfused with defibrinated blood and effect of "arterial" clamping and nerve stimulation on spleen volume and venous flow was recorded.

The volume record of the spleen before the clamp was applied either on artery or on vein was either a straight line or a slightly wavy one, showing slight rhythmic variations in volume at the rate of two per minute.

B. Results. 1. On compression and on release of the splenic artery no changes were noted in the general arterial blood pressure corresponding to those in spleen volume. As in the kidney experiments, there were sometimes a slight rise, at other times a slight fall, of the arterial blood pressure on clamping and on release of the artery, respectively.

a. Periodic spasms. There were two distinct types of periodic spasm. One, distinctly rhythmical, invariably followed the release of the compressed artery. After release of the artery the volume record rose rapidly above the control level, this initial rise being sometimes three times the fall on occlusion of the artery. Then followed rhythmic contractions and relaxations of the spleen, lasting 4 to 10 minutes, gradually diminishing in amplitude and increasing in frequency to the condition before the clamping. The second type did not invariably occur and differed from the first type in that the rhythm was less rhythmical. The increase in volume is sudden, followed by a more gradual decrease, the volume of the spleen being greater at the end than at the beginning of these spasms (filling by the portal pressure?). This size of the spleen is maintained till the end of the compression of the artery. It occurred during the first minute or two of arterial occlusion and was once observed *superimposed on the first wave of the rhythmic spasms after the release of the artery.*

b. Blood flow and spleen volume after temporary occlusion of splenic artery. Before the venous cannula was connected, the characteristic volume changes took place on clamping and releasing the splenic artery. With the cannula connected as described above and the artery released, the volume changes and the blood flow being recorded, there were six periods of marked increase and decrease in splenic volume in six minutes. At each of these periods the *venous outflow slowed or stopped during dilation and greatly accelerated during contraction of the spleen, the acceleration being greatest at the beginning of decrease in spleen volume.* It is difficult to determine to which type the periodic spasms of this experiment belong.

2. Occlusion of vein. Negative results were obtained on occlusion for 1½ minutes. In each of the 5- and 8-minute periods of venous occlusion there were rhythmic contractions for 2 to 3 minutes after release of the vein, similar to but not so marked as those described following release of the clamped artery.

3. On stimulation of the splenic nerves for 5 to 10 seconds with the blood vessels still intact, the spleen volume decreased as on clamping the artery, but after the stimulation it increased much more gradually to the control level in 8 to 15 minutes. No periodic spasms were observed following the stimulation. Increased venous flow on contraction of the spleen on stimulation of the splenic nerves was observed in all of the eleven experiments where both spleen volume and venous outflow were recorded, the increase in venous outflow being from 100 per cent to 400 per cent, greatest at the beginning of the spleen contraction, followed by slowing or cessation as the spleen dilates. After stopping venous flow by clamping the artery, stimulation of the splenic nerves is followed in a few seconds by a rapid flow of blood from the vein, in one case 35 drops in 20 seconds. A second stimulation of the splenic nerves produced a second but slower venous outflow.

Similar changes of spleen volume and venous outflow follow stimulation of splenic nerves when the spleen is perfused with defibrinated blood. The venous flow may increase 25 times during contraction of the spleen.

Discussion. The anatomy of the spleen points to several possible ways by which the spleen volume may be changed. The spleen is covered by a capsule containing smooth muscle. Trabeculae also containing smooth muscle extend from the capsule into the splenic pulp, dividing the spleen into lobes. Each lobe is supplied with a branch of the splenic artery and vein, each dividing into still smaller branches ending in thin-walled blind pouches, the ampullae. These end in blood sinuses.

It is therefore possible that change in the volume of the spleen may be brought about by 1, contraction and relaxation of the smooth muscle of the capsule and trabeculae; 2, active contraction and relaxation of the blood vessels in the spleen; 3, hindrance to flow of blood into and out of the spleen (rise in portal blood pressure, accidental pressures on artery or vein in the course of the experiment).

The last factor is at once eliminated as a possible cause of changes in spleen volume in these experiments. Accidental occlusion of the splenic blood vessels, especially the vein, were carefully prevented, and experiments were performed when the control record was horizontal. Hence only the first two factors can play a rôle in these experiments.

The mechanism of the periodic spasms during and after occlusion of the splenic blood vessels could not be determined. There were indica-

tions that the two kinds of periodic spasms noted above were produced in two different ways or rather by two different structures (splenic musculature and the blood vessel musculatures).

That the spleen may act as a heart is fully shown by the increased venous flow accompanying contraction of the spleen on stimulation of splenic nerves. That it normally does so is suggested by the spontaneous periodic contractions and dilatation following 25-minute occlusions of the splenic artery with increase and decrease, respectively, of venous flow, and by the frequent occurrence of similar spontaneous, though feeble, rhythmic contractions and dilatations, on the control record. The contraction of the spleen on stimulation of the splenic nerves is probably brought about both by contraction of the capsule and of the blood vessels.

The blood flow during known vasomotor activities in organs like the salivary gland has been shown to increase on dilatation and to decrease on contraction of the blood vessels. Hence it is probable that vasomotor activity, at least alone, was not responsible for the changes in spleen volume here noted.

It should be noted that the neuro-muscular mechanism of the spleen reacts to anemia in a manner different from that of the intestine. In the spleen there is usually paralysis or quiescence during the anemia, and marked motility on restoring the blood flow. In the intestines there is *increased* motility during the anemia and absence of motility on restoring the circulation.

IV. Intestine. *A. Special methods.* A loop of the small intestine 10 to 15 cm. long was isolated from the rest. In half of the experiments the ends were ligated, in the other half a short glass cannula was inserted at each end of the loop and held in place by a ligature around the gut. This prevented hemorrhage from the ends of the loop and established free communication between the lumen of the intestine and the inside of the plethysmograph, thereby obviating volume changes resulting from compression of gases in the loop by intestinal peristalsis. The artery to the loop was isolated for clamping before the loop itself was prepared. In two experiments the nerves to the loop were ligated and cut, the results being similar to those where they were left intact. Ten experiments were performed on four dogs.

The plethysmograph was similar to that used on the spleen.

B. Results. The characteristic curves of volume changes accompanying and following clamping the artery are shown in figure 6, curve *3*. The occlusion period is uneventful, except in the loops with the ends closed. Those records show wavy lines during occlusion apparently due to intestinal peristalsis, as stated above. After release of the occluded artery the intestinal volume changes were similar to those following release of the occluded artery in the kidney, with the exception that the

waves of relaxation and contraction were above the control level. The first dilatation and contraction lasted 2 to 3 minutes, the peak being reached within one minute after the release. This was followed by the prolonged wave of dilatation and contraction to normal, reaching this level in about half an hour after the release of the occluded artery. As in the spleen therefore there was no spasm (of the organ as a whole) comparable to that found in the kidney following temporary anemia.

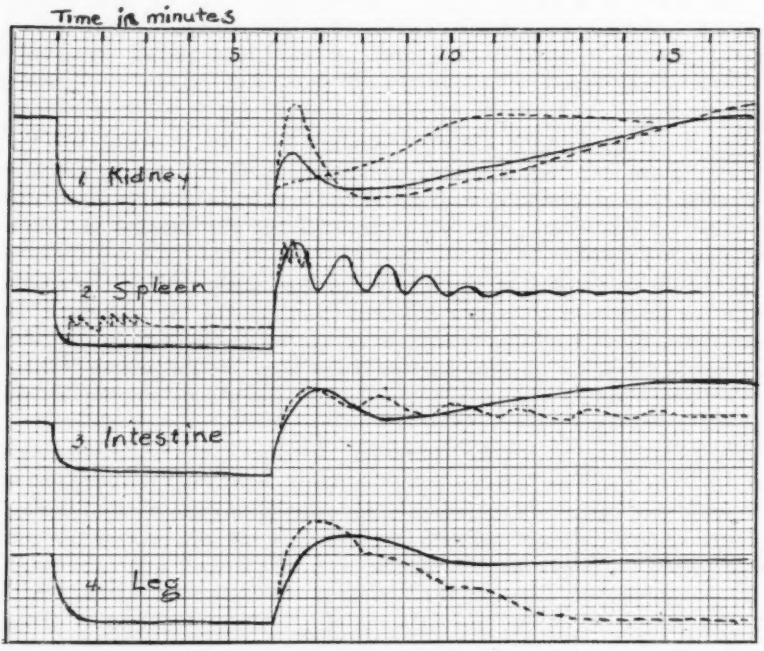

Fig. 6. Diagrams of post-anemia volume changes in kidney, spleen, intestine and leg, clearly indicating post-anemia vascular spasms in some organs (kidney, leg), while in the case of the spleen and the intestine the interpretation of the volume record is complicated by extravascular muscle tonus, and by secretions. Solid line represents the usual volume changes; dotted lines, exceptional volume changes.

On subsequent repetitions of the clamping of the artery in the same loop similar curves were obtained but the dilatations after the release were more and more pronounced and the return to normal still more delayed. In one intestinal loop the arterial clamping and release was followed by rhythmic relaxation and contraction whose frequency was similar to that observed in the spleen.

V. Leg. *A. Methods.* The femoral artery was isolated at Poupart's triangle and prepared for clamping. The leg was shaved above the knee

and slipped into an ordinary metal plethysmograph with rubber cuff. This was filled with warm tap water and connected with the recorder. Nine experiments were performed on four dogs. The artery was clamped when control volume record was steadily horizontal, and it was released after 5 to 15 minutes occlusion.

B. Results. With one exception all experiments showed no changes in arterial blood pressure corresponding with the changes in volume noted below.

The maximum fall in volume on occlusion of the artery was reached within one minute, and the occlusion period was uneventful. On release of the artery the leg promptly increased in volume to a level higher than control. In three experiments the record dropped to or almost to occlusion level in 1 to 5 minutes. In the rest it gradually lowered to or slightly below control level. The experiments were not sufficiently long to determine how long the leg vessels kept contracted in the three experiments. Rhythmic changes of volume were noted in some of the experiments for 5 or 6 minutes after the release of the artery.

VI. Thyroid. *Methods and results.* Three fairly large sized thyroids were used. Either the superior or inferior blood vessels were left intact and the rest ligated and cut. The carotid artery was ligated and cut peripheral to whichever artery was left connected with the thyroid. The same was done with the internal jugular vein. The gland was put in the glass bottle plethysmograph. The artery was compressed for 5 to 15 minutes.

The occlusion period was uneventful. On release of the artery there was prompt dilatation to or slightly greater than normal. The volume either maintained this level for 10 minutes (end of experiment) or gradually lowered to normal within that time.

In one experiment the control level was maintained for 10 minutes at the end of which the thyroid began apparently to increase in volume, at first gradually, then rapidly for at least one hour. About the time of the beginning of the rapid increase in volume it was noticed that lymph was filling the plethysmograph. There was no vein occlusion to explain the great accumulation of lymph.

SUMMARY AND CONCLUSIONS

1. Increased tone of the renal blood vessels (vascular spasm) for 3 to 45 minutes follows temporary occlusion of the renal artery for 5 to 20 minutes. This increased tone starts after release of the occluded artery. Similar increase in tone appears after releasing the occluded renal vein.

2. This increased tone is probably due to arterial blood pressure or some constituents in the blood acting as stimuli to the renal vessels rendered hyper-irritable by the anemia.

3. Anuria follows temporary occlusion of renal artery, lasting practically as long as the vascular spasm.

4. Marked rhythmic contractions and dilatations of the spleen lasting 4 to 10 minutes follow temporary occlusion of the splenic artery. Similar rhythmic changes of spleen volume, but feebler and of shorter duration, follow temporary occlusion of the splenic vein for 5 to 8 minutes.

5. A second type of periodic changes of spleen volume occasionally appear immediately after occlusion of the splenic artery and may also occur superimposed on the first type after the release of the artery. The mechanism of these periodic spasms could not be determined in these experiments.

6. The periodic volume changes of the spleen following temporary occlusion of the splenic artery are accompanied by increased venous outflow during contraction and slowing during dilatation.

7. Contraction of the spleen brought about by stimulation of the splenic nerves is accompanied by marked increase of venous outflow from the organ.

8. The intestinal volume is greater for about half an hour after temporary anemia than before the artery is occluded. Periodic increase and decrease of the intestinal volume similar to, but feebler than those noted in the spleen, occasionally follow temporary occlusion of the artery to the loop.

9. Vascular spasm in the leg probably follows temporary local anemia.

10. No vascular spasm was noted in the thyroid following temporary local anemia.

11. By the present experimental methods only the kidney (and possibly the leg) shows prolonged vascular spasm following temporary anemia. This suggests either a special highly irritable neuro-muscular mechanism in the kidney blood vessels or the presence in the other organs of conditions that mask the vascular spasm. In the spleen, for instance, there may be filling of the blood sinuses or decreased tone of the capsular muscle. In the intestine the production of secretion (paralytic secretion) may mask any vascular spasm, and the abnormally great formation of lymph may do the same in the thyroid gland.

DETERMINATION OF THE HEAT PRODUCTION IN DOGS BY THE GASOMETER METHOD

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Since Lavoisier and Laplace demonstrated the relationship between combustion and respiration, many forms of apparatus have been developed for the determination of the respiratory metabolism. The apparatus and methods used by Regnault and Reiset, Pettenkofer and Voit, Gephart and Zuntz, Magnus Levy, Dulong, Tigerstedt, Voit, Rubner, Haldane (6), Atwater and Benedict, Tissot, Lusk and Du Bois and many others, are accurate and satisfactory. Depending on the nature of the metabolic problem, sometimes one form of apparatus is preferable, sometimes another.

The open circuit method with collection of the total expired air in a gasometer of the Tissot type, and analysis of an aliquot part by the Haldane gas-analysis apparatus, is now accepted as the most exact method in short experiments for the simultaneous determination of the respiratory quotient and heat production. The details of the technic of this method for man have been fully described by Boothby and Sandiford (3); certain modifications, however, have been found necessary for the accurate determination, in dogs, by the gasometer method, of both the respiratory quotient and the metabolic rate in succeeding short periods.

Before metabolism studies are undertaken, the dogs are kept for a time in large kennels at the laboratory farm until they presumably have acquired immunity to distemper. They are there fed a mixed diet, no attempt being made to regulate their weight. After being brought to the laboratory, the animals are kept in individual cages and are liberated daily while the cages are being cleaned. At 2:00 p.m. they are fed a diet of biscuit, with a calorie content sufficient to cover the maintenance requirement as calculated from tables by Dechambre (5). Possibly a better way to compute the amount of food required to maintain a constant body weight is to observe the animal for a few days, determining the amount of food actually eaten during twenty-four hours, and weighing the animal

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at the same hour each morning, varying the amount of food supplied as the weight curve goes up or down. In a very few days it is usually possible to determine the maintenance requirement.

The food used during the periods of observation was dog biscuit which contained, on analysis, approximately 22 per cent protein, 4.5 per cent fat, 62 per cent carbohydrate, with small amounts of water, and a caloric content of 3.85 calories for each gram. This mixture is slightly lower in fat than that used by Lusk but is readily eaten by the animals and keeps them in good condition. Occasionally, a little whole milk is allowed in place of some of the biscuit.

Not all human beings are suitable for metabolic studies, neither are all dogs. Suitability depends on the individual. One cannot work with animals and not realize that they are not simply mechanisms but are individuals with their own peculiar reactions and characteristics. Generally, we have found that dogs with a bull-terrier strain accept the training more philosophically, and seem to realize the necessity of remaining quiet more readily than do the nondescript breeds. For convenience in handling, their weight is preferably between 10 and 18 kgm.

Before being used for experiments, the animals are carefully trained by a laboratory assistant. In the first period of training the dog is placed on the table for an hour or less, with feet held in place by restraining straps. In the second period the mask is put on without being adjusted tightly, and in the third, the mask is placed in position, the neck is wound with a rubber dental dam in the usual manner, and the dog is left on the table for a much longer time. These steps, of course, are dependent on the animal's behavior; if he is obviously bad-natured he is discarded immediately. Success in training the animals depends largely on the patience and discretion of the trainer, and a "firm but gentle hand" is a necessary prerequisite. An animal may be spoiled for further work either by pampering or rough treatment. During our earlier experiments it was found that the animals became very much excited on being taken from the cage, apparently because of the novelty of being out of the cage. Therefore, the animals were allowed a few minutes' liberty each day. As anticipated, this seems to reduce the excitement incident to their being handled experimentally.

A Tissot gasometer, as modified by Boothby and Sandiford, is used; it is equipped with rubber flutter valves arranged so that the expired air is collected in the gasometer. The air inspired is always taken directly from outdoors.

The animal is placed on its side on a "rack," which in turn is supported by a strongly built wooden table. The rack is about 39 inches long and 20 inches wide, and the upper surface is composed of thin strips of wood 1 inch wide, and 0.5 inch apart. The two lateral edges of the rack are

raised 3.5 inches above the level of the center. This divides the surface into two planes, each having a gradual slope toward the center where the angle is converted into a shallow groove by a strip of tin 6 inches wide. An animal seems to lie more comfortably on this slope than on a flat surface.

The mask is a hollow cone of thin copper sheeting, open only at one end and made so the fold of the seam is on the inside, leaving the outside

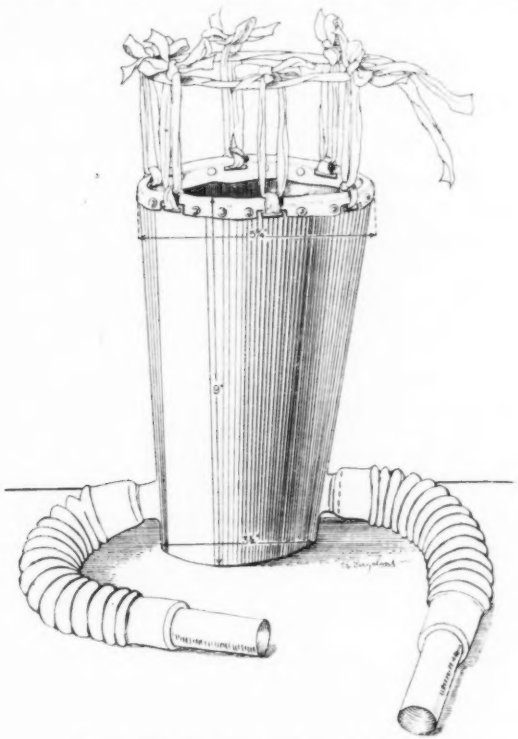

Fig. 1. Metabolism mask used for dogs

perfectly smooth and even, without the slightest furrow (fig. 1). To the closed end are attached two short metal tubes, 1 inch in diameter and 1.5 inches long. These are soldered in place on opposite sides and attached to each is a short (10 inch) length of corrugated, canvas-covered rubber tube to make connections with the gasometer tubing. The edge of the open end of the mask is covered with folded leather, and there are five or six holes in the copper, arranged at intervals around the circumference, to which are attached 12-inch lengths of narrow cotton tape.

A roll of rubber dental dam about 10 feet long and 5 inches wide and of medium weight is used to make an air-tight connection between the mask and the dog's neck, as will be described later.

Two tambours are placed in position, one on the animal's abdomen and one on the chest. These are connected with writing points and a kymographic record of the respiration is made during the period of the experiment. The tambours, being in close proximity to the limbs, record each movement of the animal, and thus the tracing shows not only alterations in the character and frequency of the respiration, but also indicates the degree of muscular activity.

At 5:00 p.m. on the day preceding an experiment, any food remaining from the daily feeding is taken from the animal's cage. Before an experiment, which usually commences at 8:00 a.m. after a fifteen-hour fast, the animal is allowed a few minutes' freedom in a large outdoor cage where he usually empties bladder and rectum. This is of special importance if the experiment planned is of long duration, as the discomfort of a distended bladder invariably causes restlessness. The animal's weight is recorded in the laboratory and he is placed on the table, being handled as little as possible. He is made comfortable by placing small cotton-filled pads between him and the surface of the rack. When he has assumed what seems to be the position of choice, the feet are tied loosely to the rack by means of soft canvas straps. It should be emphasized that the tying will not prevent movement, but it will lessen the desire to shift position which, in the absence of a restraining band, is apparent even in the best-trained animals. As much importance should be attached to an attempted movement as to a completed one in determining whether the test is satisfactory. For safety a canvas strap, 2 inches wide, is usually placed loosely across the chest, in no way impeding respiration. However, the trained animal will lie perfectly quiet without restraint.

A fairly wide, strong cotton tape is next tied rather loosely around the animal's neck and the mask is placed over the head. The tapes on the latter are tied to that around the neck, care being taken that there is a reasonable space (1.5 inches) between the tip of the animal's nose and the end of the mask. The trained animal shows no aversion to this procedure. The next step is the application of the rubber bandage. The dog's neck, which has been closely shaved, is moistened, and the rubber bandage is thoroughly wet by placing it in water. It is not necessary to use vaseline. After fastening by several turns around the mask, the wet rubber bandage is passed smoothly and snugly around the neck two or three times, then brought back up on the mask where the end is secured by a narrow gauze bandage. The rubber bandage must be applied evenly to the neck, using as few turns as possible, but firmly

enough to prevent backward leakage of expired air and yet not cause any respiratory or circulatory embarrassment. This requires some care but can readily be accomplished if the animal's neck is kept closely shaved. The latter is very important, for with several days' growth of hair on the neck, one is likely either to adjust the rubber too firmly and produce respiratory difficulty and venous congestion, or else too loosely and allow a leak. On the other hand, when the bandage is well applied the animal breathes normally, and there is no evidence of venous stasis. After the mask is adjusted, the dog's head is laid in the position which seems most natural, and is fastened to the rack by a stout leather strap. The dog should be carefully watched for evidences of discomfort, such as apparent difficulty in breathing, repeated attempts to shift position, or frequent swallowing. Frequent swallowing usually seems to be a sign that the animal is uncomfortable and although he may be very quiet otherwise, his rate is almost invariably higher than normal. In such cases readjustment of the rubber dam or slight shifting of the animal's position will usually suffice to remedy the discomfort.

There is always a preliminary period of one hour before any actual tests are run. We have noted, on several occasions, that a dog's temperature rises 1°C. or more, from the excitement and exertion of being out of its cage for a few minutes. The temperature, however, returns to normal after about forty-five minutes of quiet. The records of tests run after the animal has been on the table an hour are invariably lower than any run before that time, and sometimes higher than those following, even though the animal may have been perfectly quiet during the whole preliminary period. These observations emphasize the importance of a protracted preliminary period.

In experiments on the effect of foodstuffs, it is sometimes necessary to introduce the material by stomach tube. This procedure, which is surprisingly well tolerated, is carried out before the animal is placed on the table for the preliminary period.

Body temperature (rectal) is recorded at the end of the preliminary period and again at the end of the experiment. There should be no appreciable change but sometimes a drop of as much as 0.8°C. occurs in the course of a three-hour period, if the environmental temperature is too low or the dog is not sufficiently protected by blankets.

Before commencing a test, the gasometer is rinsed three times with expired air to make certain that the dead-space of the mask and apparatus is filled with expired air. Gasometer readings and duration of tests, usually from ten to fifteen minutes, are checked by two observers. Samples of the expired air are collected over mercury; duplicate, and in the crucial experiment, triplicate analyses are made on the Haldane apparatus; the calculations of the results are carried out and checked as described by

Boothby and Sandiford (3). From the data, the total number of calories, for each hour is calculated, and also the number of calories for each hour for each square meter of body surface, the latter being determined by Rubner's constant in Meeh's formula: $11.2\sqrt[3]{(\text{weight in grams})^2}$.

At the conclusion of an experiment, the tightness of the mask is always tested in the following manner: without changing the position of the animal, the juncture of bandage and neck is liberally covered with thick soap-suds, and the animal is allowed to breath against the uncounterpoised weight of the gasometer bell. Any backward leaking of air is readily demonstrated by a bubble; only very rarely, however, does a leak occur. This procedure would perhaps be better done before the experiment is started were it not for the excitement caused by this additional handling. In cases in which there is doubt as to the "fit" of the mask it is best to test both before and after the experiment. Recently, the expiratory tube has been placed in a bottle of water for a distance of 10 to 15 cm. to produce a greater and more readily measured resistance.

During a test, the animal is always closely watched by the observer and any movement or sign of restlessness noted. Pulse and respiration rates are counted frequently by a stethoscope applied lightly to the chest. Any increase in pulse or respiration without any apparent reason, even though unaccompanied by movement, is considered significant, and the validity of the test questioned before the results of the air analysis are known. Careful observation is considered the best means of determining whether basal conditions obtain, and it is therefore always decided at the time of an experiment whether the test is satisfactory. The judgment of the observer guided by the kymographic record is, as a rule, confirmed by the results of the test.

Even though a test may be considered unsatisfactory, it is always carried to the calculation of the heat production, so that it may be compared with the results of satisfactory tests on the same animal. By repeated comparisons one obtains a fair idea of the relative importance of certain movements in elevating the metabolic rate, and how much movement, if any, is compatible with a normal rate. Some dogs, after they have been on the table for a time, almost invariably exhibit certain twitching movements of fore or hind limbs. Although the animal does not seem to be asleep, these appear to be involuntary and are usually unaccompanied by a rise in pulse rate. Repeated observation has shown that such movements, when not accompanied by acceleration of pulse, do not materially affect the production of heat and, when in moderation, are not considered as invalidating the results. While it is impossible to write an equation or construct a formula by which to judge whether a test is satisfactory, one can, nevertheless, predict with reasonable accuracy the effect of an animal's behavior on the metabolic rate. Unless

movements or errors in technic occur, the various periods should show close agreement as illustrated in figure 2. The temperature of the room is kept fairly constant, and usually between 22° and 25°C., so that the heat production of the animal is not greatly influenced by environmental temperature. It has been shown by Rubner that the heat production of a dog remains practically unchanged if the room temperature is kept between 20° and 30°C. However, if the temperature is below 25° it is necessary to cover the dog with a light blanket, as careful observation shows that unless this is done, occasional periods of slight shivering occur which elevate the metabolism. However, we have not been able to maintain a constant temperature in any way comparable to that in Lusk's experiments with the dog in the respiration calorimeter. As emphasized

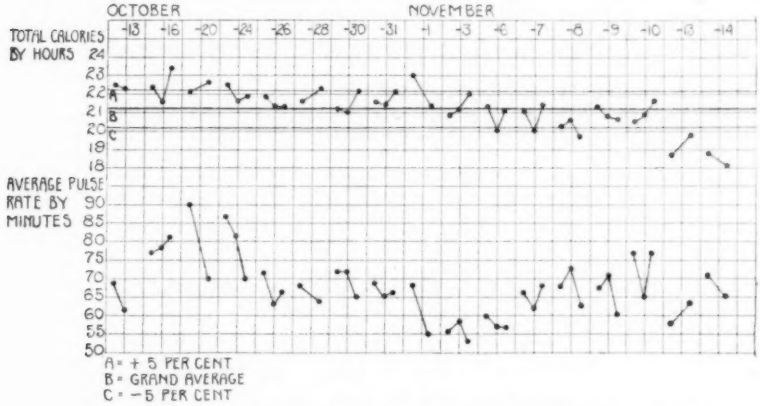

Fig. 2. Chart showing constancy of daily metabolism in dog F 754

by Rubner, the significance of many experiments is invalidated by neglecting to eliminate the rise of metabolism due to too low environmental temperature.

In some of the earlier experiments on the calorigenic action of adrenalin, attempts were made to anesthetize the animals with urethane. Doses of from 0.5 to 1 gram of urethane for each kilogram of body weight were given in aqueous solution by stomach tube and also by rectum, with very unsatisfactory results. Some animals, sensitive to the drug, would be deeply influenced by it, others would vomit or expel the rectal injection and remain in an excited condition unless more of the drug were administered, when they might succumb to an overdose. Even when deeply under the urethane, however, excessive flow of saliva made them poor subjects for experimentation and necessitated the occasional removal of the mask.

In an attempt to obtain the average temperature of normal dogs, the rectal temperatures of eighty-three animals, selected at random, irrespective of size or length of hair, were taken. All, however, were being kept in the same environmental room temperature and surroundings, and maintained on the same type of mixed diet, and were apparently in good health. The temperatures ranged from 37.7°C. to 39.9°C., the majority being between 38° and 39°C. as follows: below 38°C., 3 per cent; between 38° and 39°, 80 per cent; above 39°, 17 per cent. The average rectal temperature of the eighty-three dogs was 38.7°C. Variations in temperature beyond these limits are therefore an indication of some illness and no experiment should be performed upon the dog that day.

In order to obtain, if possible, an approximate average heat production for normal dogs, the results of 254 observations on thirteen animals are summarized. Eight of the dogs had short hair, and five had long hair. The range in weight was from 10.0 to 17.7 kgm., although nine of the

TABLE 1

Summary of the results of heat production of dogs, irrespective of sex

LENGTH OF HAIR	DOGS	SATISFACTORY TESTS	TOTAL CALORIES FOR EACH HOUR	AVERAGE CALORIES EACH HOUR	
				Each kilo	Each square meter
Long	5	70	22.6	1.82	37.5
Short	8	184	25.4	1.91	40.3
Total	13	254	Ave. 24.3	1.87	39.2

animals weighed between 10 and 15 kgm. All were young adult dogs, in good physical condition, and at least five satisfactory observations were made on each animal included in the averages (table 1).

There appears to be a tendency to a slightly greater heat production in short-haired animals. This is in accord with the observation by Rubner that the heat production of a small dog was increased when he was shaved. It is possible, however, that the difference between the long- and short-haired dogs would disappear, provided the experiments on the short-haired dogs were repeated at a higher room temperature or if the animals were more warmly covered. At present we are inclined to interpret this finding as a criticism of our technic rather than as an indication of a greater basal heat production.

Further comparison of the production of heat by the animals shows that the females averaged a little higher per square meter than the males. In the long-haired dogs the average for males was 37.4 calories and for females 37.5 calories; in the short-haired group the average for the males was 37.0 calories and for the females 42.2 calories.

TABLE 2

The average basal metabolism as determined by 254 observations on thirteen dogs

DOG	DESCRIPTION	SATISFACTORY TESTS	WEIGHT, KGM.	TEMPERATURE, DEGREES C.	PULSE	RESPIRATION	SURFACE AREA	TOTAL CALORIES EACH HOUR	AVERAGE CALORIES FOR EACH KILOGRAM	CALORIES FOR EACH KILOGRAM			AVERAGE CALORIES FOR EACH SQUARE METER	CALORIES FOR EACH SQUARE METER		
										Range		Per cent variation of average from grand average		Range		Per cent variation of average from grand average
										Highest	Lowest			Highest	Lowest	
E381	Young adult male, medium long hair	8	10.0	38.5	63	21	0.52	18.7	1.86	2.25	1.50	−1	35.9	43.1	30.0	−8
E460	Young adult female, short hair	5	15.9	38.5	83	22	0.71	33.1	2.09	2.38	1.89	+12	46.9	53.4	43.0	+20
E789	Female, medium long hair	8	11.2	38.6	82	15	0.57	23.7	2.12	2.43	1.90	+13	42.3	47.2	38.4	+8
E850	Female, short hair	8	10.0	38.4	100	9	0.52	24.9	2.52	3.01	2.27	+35	48.0	55.3	44.1	+22
F188	Male, short hair	4	16.4	38.3	82	15	0.72	30.1	1.84	1.93	1.79	−2	41.8	43.9	40.6	+7
F368	Female, medium short hair	7	11.0	38.5	76	18	0.55	21.1	1.91	2.01	1.78	+2	38.2	40.5	35.4	−3
F5	Female, short hair	28	16.1	38.7	88	25	0.71	31.6	1.98	2.40	1.46	+6	44.5	53.7	34.1	+14
F383	Female, medium long hair	6	12.7	38.4	45	25	0.61	21.2	1.68	1.97	1.47	−10	34.8	41.0	30.3	−11
F515	Male, medium long hair	10	11.8	38.7	48	33	0.58	22.5	1.91	2.08	1.69	+2	38.8	42.7	34.6	−1
F513	Male, short hair	61	12.6	37.8	65	12	0.61	21.4	1.70	1.94	1.50	−9	35.3	40.0	31.3	−10
F551	Male, short hair	7	12.7	37.9	60	45	0.61	20.6	1.63	1.90	1.50	−13	33.9	39.5	31.3	−14
F257	Female, long hair	38	17.7	38.5	64	15	0.76	27.0	1.53	1.79	1.35	−18	35.5	41.3	31.1	−9
F754	Female, short hair	64	12.6	38.0	66	13	0.61	20.2	1.60	2.02	1.27	−14	33.4	41.0	26.7	−15
Average								24.3	1.87				39.2			

It will be seen from table 2 that, although some of the individual animals show fairly wide variations from the grand average of 39.2 calories for each square meter each hour, seven of the thirteen (55 per cent) are within plus or minus 10 per cent of this figure, while eleven of the thirteen (85 per cent) are within limits of plus or minus 15 per cent of the average. The average heat production of 39.2 calories for each square meter is almost identical with the Du Bois standard for human beings, and is confirmatory evidence of the theory that heat production under similar conditions of age and sex is proportional to the surface area.

However, these averages are only of theoretic interest, since the nornal heat production of each animal used must be carefully determined under uniform conditions of maintenance and environment before any experimental data are collected. In this connection, it must be borne in mind that when the laboratory animal is confined in a cage, the metabolism probably tends to fall, as pointed out by Lusk (7), (8). This fact, as well as the constancy of the daily metabolism, is well illustrated in figure 2. The experiments of the last two days are, however, so far below the average that the possibility of a leak, with loss of some of the expired air, must be considered.

No consistent variation in the production of heat was evident between the large and small dogs studied on the basis of either calories for each kilo or calories for each square meter; the variation in size, however, was not great as the smallest dog weighed 10.0 kilos and the largest 17.7 kilos.

CONCLUSIONS

1. The technic described offers a simple and inexpensive method of determining, in dogs, the respiratory quotient and the heat production over many successive short periods.

2. The method makes possible certain experiments which could not be carried out with the closed calorimeter, for example, the investigation of the calorigenic action of adrenalin (4), the study of the metabolic curve after removal of the liver (9) and the effect of the intravenous injection of glucose, as well as other problems which necessitate certain manipulations coincident with the recording of the metabolic rate.

3. The heat production of a normal dog is more closely proportional to the surface area, as calculated by Meeh's formula, $11.2 \sqrt[3]{\text{weight in gms.}^2}$ than to body weight. The average obtained of 39.2 calories for each square meter of body surface is remarkably close to that found by Du Bois for human beings.

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SOME OBSERVATIONS ON LYMPH-PRESSURE

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When the general physiology of the lymph system is reviewed it is at once apparent that the preponderance of investigation has dealt with those factors that enter into the formation of lymph; comparatively few studies, on the other hand, have been made of the forces that drive the lymph through its channels. This disparity in treatment is brought into stronger relief when the general physiology of the blood vascular system is considered; for in this system blood-pressure has always aroused the keenest interest, whereas those processes giving rise to blood-plasma formation have not been so extensively investigated. The reasons for these discrepancies lie mainly in the anatomical arrangement of these two vascular systems. It is with the view of possibly throwing some additional light on the pressure-changes in large lymph vessels, that the following observations on thoracic duct pressure are given.

METHOD. The animals used in these experiments were nine dogs, between 12 and 18 kgm. in weight, which had previously served in a course in operative surgery conducted by Dr. E. C. Cutler, and had been subjected to one or two of the three following operative procedures: posterior gastro-enterostomy, end-to-end anastomosis, and lateral anastomosis of the small bowel. In all cases the dogs had completely recovered from their last operation which was performed more than forty-five days previous to these experiments. Furthermore, in every instance a post-mortem examination showed that the operations had been successful, and that the various anastomoses showed no evidence of leakage or obstruction. All food, with the exception of water, was withheld for the twenty-four hours preceding the experiment described below.

The object of the dissection was to secure a preparation in which the uncontaminated thoracic duct lymph could be made accessible for pressure observations. The animal was etherized by an intratracheal insufflation method in which a constant amount of air mixed with various amounts of ether vapor was forced into the lower part of the trachea at a pressure not exceeding 10 mm. Hg. The dog was placed on his back and an incision was made commencing at a point half way along the posterior border of the left sterno-cleido-mastoid muscle, extending

inferiorly and slightly medially, then laterally to a plane bisecting the superior fibers of the pectoralis major muscle, and stopping at the anterior axillary line. The dissection was carried down to the external jugular vein, through the pectoralis major and superior fibers of the pectoralis minor muscle until the axillary vein was reached. All the bleeding points were carefully ligated. From this point blunt dissection was carried on with scissors in a central direction along the external jugular and subclavian veins. It was found expedient to follow along the medial border of the external jugular vein until a white fascia, spoken of as the suspensoria pleurae and described as a continuation of the deep cervical fascia, was reached. A good way to pierce this fascial plane was observed to be to follow closely along the medial and anterior aspects of the external jugular vein, and to alternate with a dissection along the anterior and inferior portions of the subclavian vein, for it was evident that one of the commonest accidents, namely, rupture of the pleura, occurred when the veins were not followed carefully. As soon as the innominate vein was seen, a free passage immediately surrounding it was obtained. Ligation of the various vessels was then undertaken in a definite order. In the first experiments the subclavian artery was ligated in order to avoid not only a high venous pressure in the subsequently ligated subclavian vein, but also to eliminate any excess of lymph formation from the limb because of the venous ligation; later the elimination of all lymph trunks except the thoracic duct made the ligation of the subclavian artery unnecessary. The transverse scapular artery and vein were cut separately between ligatures. The veins were ligated in this order: *1*, internal jugular, including usually the left tracheal lymph trunk; *2*, subclavian; *3*, external jugular; *4*, innominate. The reason for this sequence of ligation was to prevent blood from forcing its way into the thoracic duct under relatively great pressure and thus giving rise to unnatural conditions. In a few moments the lymph which was being continually poured into the venous angle distended the venous junction and also showed the course of the thoracic duct more distinctly. The innominate vein was cut between ligatures in order to afford a better exposure for disssection at the central end of the subclavian vein; frequently a small vein coursing over the first rib was seen to enter the subclavian vein at the venous angle, and for the ligation of this vein preliminary section of the innominate vein was found necessary. Slight traction on the peripheral stump of this vein was found convenient and it was transfixed to prevent the ligature from being pulled off. Since the thoracic duct was clearly seen, ligation of all tissues, particularly those suspected of harboring neck lymph vessels, was carried out; furthermore, the fatty areolar tissue directly posterior to the venous angle was also ligated.

This dissection provided a Y-shaped preparation in which the subclavian and external jugular veins represented the two arms, while the innominate vein was the stem. In some cases the ligatures between which the innominate vein was cut were tied, and a more natural position for the venous angle obtained; but no particular advantage in this procedure was noticed. Into this venous angle the thoracic duct emptied as a single vessel or, as was about equally found, as two trunks; naturally the venous junction was greatly distended with lymph. A small glass cannula was inserted into the subclavian vein, and a large, squared-off cannula which was an arm of a T glass tube of 3 mm. internal diameter was inserted into the external jugular vein. All the glass tubing with which the lymph was to come into contact had been coated with a thin layer of paraffin. The cannula in the subclavian vein served as a means for washing out the preparation; an 8 per cent aqueous solution of sodium citrate led from a burette was used for this purpose. It was not necessary to wash out the venous angle oftener than once an hour, and frequently readings extending for almost two hours were made with the lymph still liquid in the preparation (see fig. 7). To that arm of the T-tube opposite to the cannular portion was attached a short piece of rubber tubing through which the lymph was collected, or when this tube was clamped, the lymph was forced up the stem of the tube. This stem was lengthened by the addition of a glass tube of the same diameter and thickness as that of the T-tube, and was attached to the latter by means of a short glass cuff sealed with paraffin. The stem, marked off in centimeters so that the height of the lymph could be immediately read off, was kept in a vertical position by a clamp attached near its distal end. In some cases it was found necessary to break down either with a blunt probe or a fine pair of scissors the bicuspid valve usually situated at the junction of the external jugular vein with the subclavian; often the proximity of the cannula alone served to abolish the function of this valve.

To study and record finer changes in lymph-pressure, the stem of the T-tube was connected to a delicate manometer[1] of which the writing arm traced changes in lymph-pressure on a kymograph. Often the blood-pressure as registered from the femoral artery by a mercury manometer; respiration, and second intervals were recorded simultaneously with changes in lymph-pressure

Observations. When the lymph was allowed to flow from the arm of the cannula to which the rubber tube was attached it was seen that

[1] This manometer was kindly lent by the Department of Pharmacology of the Harvard Medical School where it was designed and built by Mr. H. George. The principle is essentially that of a mercury manometer with kerosene in place of mercury, having air conduction from the animal to the manometer, and employing a carefully balanced writing lever.

the curve registered on the drum from pressure changes in the stem of the cannula showed a series of more or less regular waves. For example, in the graph shown in figure 1, there are ten distinct waves occurring at the rate of one wave every 17.5 seconds. It was noticed that these waves represented the individual drops of lymph as they came from the cannula, and that the crest of each wave marked the moment at which the drop actually fell. In this respect the graph not only recorded fluctuations in lymph-pressure but also the amount of lymph escaping from the cannula under ordinary conditions. It was furthermore evident that the lymph-pressure tracing was composed of a series of small sharp fluctuations, and in order to study these the speed of the drum was suddenly increased.

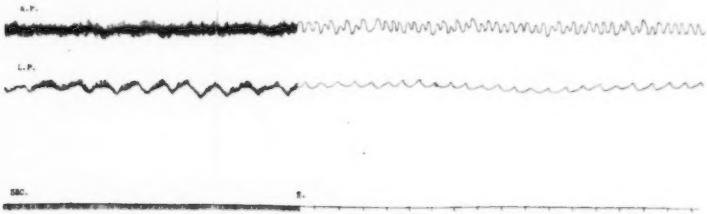

Fig. 1. Dog LP3. Simultaneous record of blood-pressure, lymph-pressure and time to show the characteristic changes in lymph-pressure in a cannula introduced into the external jugular vein when the lymph escapes. The larger waves to the left represent individual drops of lymph. The speed of the recording drum was suddenly increased at *S* in order to show the nature of the small waves making up the lymph-pressure curve. These individual waves represent pressure changes due to respiration. *A.P.*, blood-pressure in the left femoral artery; *L.P.*, lymph-pressure in the left external jugular vein; *SEC.*, time recorded in seconds.

As a result the small fluctuations revealed themselves as definite waves of regular size and shape occurring at regular intervals and at the rate of 75 per minute. A close examination showed that these small waves were not smooth in outline but had certain superimposed elevations and of these there were two definite ones for each wave. The number of these elevations in a given interval averaged 90 per cent of the number of arterial pressure waves in the same interval; these oscillations in lymph-pressure represented in all probability changes due to the recording instrument rather than to pulse pressure. However, inspection of a rising column of lymph showed that there was a definite rise with each expiration, and that each heart-beat showed a slight fluctuation.

In order to demonstrate the relationship between lymph-pressure changes and respiration, a graph shown in figure 2 was taken. In this case the lymph out-flow tube was clamped, and the lymph compelled to rise in the stem of the cannula. As a result the writing-lever for lymph-pressure was forced downward in proportion, within certain limits, to

the rise of lymph in the cannula. It was seen that the wave of lymph-pressure corresponded exactly with the respiratory wave, and that the lowest point in the lymph-pressure curve (which represented the greatest amount of pressure) corresponded to the trough in the respiratory wave curve which signified expiration. In other words, the peak of lymph-pressure was reached at the end of expiration. Furthermore, the lymph-pressure curve paralleled to a remarkable degree the respiratory curve; for there was a slight sharp fall in lymph-pressure corresponding to the comparatively short period of inspiration, followed by a slowly increasing rise in lymph-pressure synchronous with the relatively long expiration.

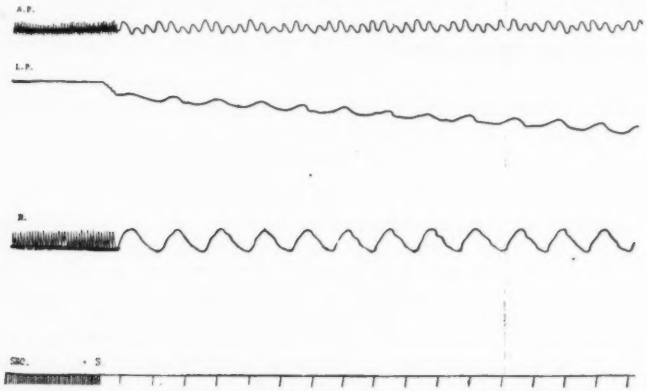

Fig. 2. Dog LP5. A simultaneous record of blood-pressure, lymph-pressure, respiration and time, to show the relation between the waves in the lymph-pressure and respiration curves. The out-flow tube for the lymph was clamped immediately before the speed of the drum was increased at *S*. The downward movement in the lymph pressure graph represents an increase in lymph pressure, and the up-stroke in the respiratory curve signifies inspiration. *A.P.*, blood-pressure in the left femoral artery; *L.P.*, lymph-pressure in the left external jugular vein; *R.*, respiration; *SEC.*, time in seconds.

To record graphically the rise in lymph-pressure, the tracing in figure 3 was taken. In this case the lymph out-flow tube was again clamped, and as the lymph-pressure rose the writing-lever descended. The bottom of the curve did not mark the peak of lymph-pressure, but the record was stopped because the writing-lever for pressure threatened to interfere with the time record. It will be seen that a series of waves, again indicating drops of lymph, characterized the first portion of the curve. After the out-flow tube was clamped the lever descended showing a definite wave for each respiratory movement. At the middle of the curve the fluctuations were less because the writing point was applied too closely

to the drum. The lowest portion of the curve indicating relatively high pressure still showed variations with each respiration.

In order to learn how much pressure could be put on the thoracic duct to force its contents into the venous angle under ordinary conditions the out-flow tube was clamped and the rise of the lymph in the stem of the cannula was noted at regular intervals. Figure 4 shows a curve typical of the gradual rise in lymph-pressure. It was noticed that

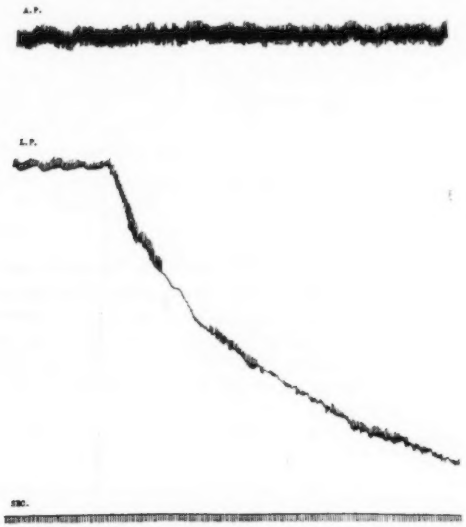

Fig. 3. Dog LP3. A simultaneous record of blood-pressure, lymph-pressure and time to show graphically the rise in lymph-pressure. The first part of the curve shows large waves indicating drops of lymph. Immediately after the out-flow tube was clamped the lymph-pressure rose and was recorded by a downward movement of the lever. The small steep waves are due to respiratory variations. *A.P.*, blood-pressure in the femoral artery; *L.P.*, lymph-pressure in the left external jugular vein; *SEC.*, time in seconds.

after it reached its peak the pressure invariably receded a fraction. Furthermore, to ascertain whether this type of curve would repeat itself, the pressure was reduced to zero by unclamping the rubber tube and a new pressure record obtained. The curve thus obtained is given in figure 5, and it differed from the curve in figure 4 mainly in that it rose faster and reached a level which was either a little below or a little above the previous level. Some of the factors bearing on this difference will be discussed subsequently.

An experiment was then undertaken to see for what length of time such a pressure record could be obtained, and also whether it could be influenced

after reaching its peak. Such an attempt is pictured in figure 6. The first portion of this curve resembles to a striking degree the curve in figure 4. At *S* the left groin was shaved for the subsequent intravenous administration of pilocarpin at *P*. A sudden rise ensued which was allowed to

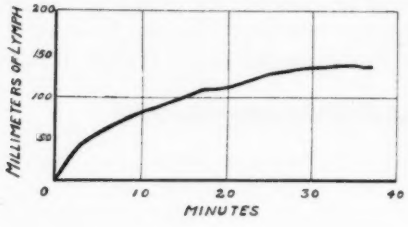

FIG. 4

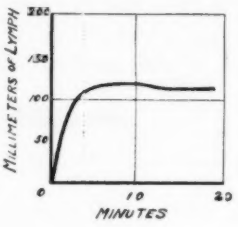

FIG. 5

Fig. 4. Dog LP4. A typical curve to show the gradual increase in lymph-pressure in the thoracic duct under ordinary conditions. Observations were made every minute. Ordinates represent the lymph-pressure in the left external jugular vein in millimeters of lymph; abscissae give the time intervals in minutes.

Fig. 5. Dog LP4. A curve taken immediately after data for figure 4 to show the early quick rise. Same notation as for figure 5; to be compared with that figure.

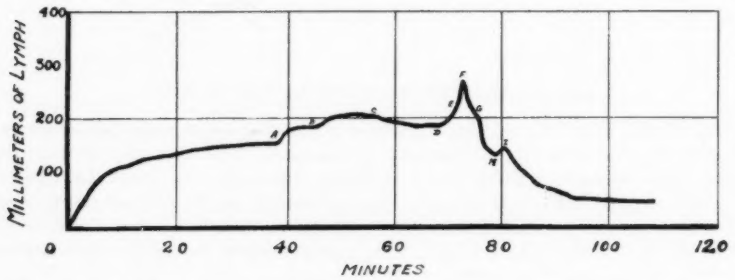

Fig. 6. Dog LP5. A curve to show the effects of various procedures on lymph-pressure. Ordinates represent the lymph-pressure in the left external jugular vein in millimeters of lymph; abscissae give the time intervals in minutes. *A.*, right groin shaved; *B.*, shivering; *C.*, respiration recorder removed; *D.*, 14 mgm. pilocarpin nitrate injected into right femoral vein; *E.*, rapid respirations; *F.*, respirations ceased; *G.*, slight changes in lymph-level due to heart-beat; *H.*, a few spasmodic respirations; *I.*, respirations ceased.

come to rest before the pilocarpin was given. Soon after the administration of pilocarpin had begun, respirations increased in number and degree, the lymph-pressure mounted to a height which exceeded the length of the stem of the cannula, and after an extension was hastily

provided reached a height of 270 mm. Respirations ceased thirty seconds later and the lymph-pressure thereupon gradually fell until it reached 50 mm. at which level it remained for seven minutes. After being released the lymph was still limpid although this one observation had extended over a period of one hour and forty-eight minutes.

To try, finally, for greater heights to which the lymph-pressure might mount, the animal was subjected to labored breathing by the administration of large quantities of ether. This record is given in figure 7. It was noticed that the lymph-pressure rose rapidly, reached 350 mm. and stopped suddenly after respirations had ceased.

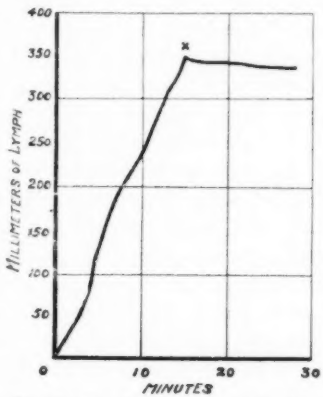

Fig. 7. Dog LP6. A curve to show the rapid rise of lymph-pressure due to deep respiratory movements before death. Ordinates represent the lymph-pressure in the left external jugular vein in millimeters of lymph; abscissae give the time intervals in minutes. *X*, cessation of breathing.

Discussion. A review of the literature on the subject of pressure in large lymph trunks has revealed only a few reports of which the earliest were made more than sixty years ago. The first was in 1850 by Noll (7), working in Ludwig's laboratory, who used the cervical lymph trunk of dogs mainly, and employed in one case (exper. 5) the corresponding lymph trunk in a cat. The lateral pressure in the lymph vessel, measured in terms of centimeters of a soda solution as read from a manometer having a diameter of 3.5 mm., was found to vary between 8 and 18 mm. of soda solution; in one experiment (exper. 1), however, the solution reached a height of 26 mm.[2] He noticed, furthermore, that the lymph-pressure rose during expiration and fell during inspiration, and that it was increased by any peripheral pressure, such as stroking. He did not believe that respiration had much influence on pressure, and that the chief factors in lymph propulsion were the valves, the elasticity of the vessel walls, and a small intrinsic pressure (vis a tergo).

The other early report on lymph-pressure was made originally as a dissertation in 1860 by Weiss (8) but subsequently the work appeared in Virchow's Archives in 1861. Weiss used the cervical lymph trunk chiefly in colts weighing 230 pounds, employing essentially the same kind of apparatus previously described by Noll, except that the diameter of the cannula was 1.5 mm. He found that the lateral pressure in the cervical

[2] The pressures of 14 to 26 mm. Hg frequently cited (Colin, Jappelli) as found by Noll are erroneous.

lymph trunk of the dog varied from 5 to 20 mm. of a soda solution having a specific gravity of 1.080. The lateral pressure in the thoracic duct of a colt he found to be very much higher, namely, 15.74 mm. Hg and he made five tracings on a kymograph to show the changes in lymph-pressure under ordinary conditions. He furthermore determined that the lymph-flow in the thoracic duct of the colt varied from 84.2 to 185 grams per kilogram of body weight per day.

It is obvious that these two investigators were interested primarily in the pressure within large lymph trunks under ordinary conditions, and that the immediate forces giving rise to this pressure were of secondary importance. Both recognized that expiration raised lymph-pressure, and Weiss noticed that even inspiration would raise the pressure because the force of muscle contraction overbalanced the negative pressure in the thorax. To Weiss again belongs the credit of being the first one to record on a drum changes of lymph-pressure. Weiss was definitely hampered by the unsatisfactory anesthesia following the intravenous administration of tr. opii, for he frequently referred to the difficulties encountered in keeping the animals quiet during the experiment, and there is no doubt that this factor caused the irregularities in the curves. The cannula itself, the occasional clots of lymph, the variability of the thoracic duct entrance into the veins particularly in view of its frequent branching, are factors which also affected the determination of absolute lymph-pressure. However, the lymph-pressure obtained agreed very well with the venous pressure determined for the external jugular vein, and there is no reason to expect a higher lymph-pressure. The only discordant note in this respect is the comparatively high pressure of 15 mm. Hg found in the thoracic duct of the colt; for if one assigns 1 mm. Hg as an average pressure in the external jugular vein, thus doubling the value as found for the dog, there remains a difference of 14 mm. Hg which must be necessary to drive the lymph into the venous angle of the colt, if the work of Weiss is accepted. Certainly all investigators who have ever opened the thoracic duct in the neck of dogs have never noticed lymph escaping under such great pressure, nor have those surgeons who have accidentally incised the thoracic duct in the neck in man reported any remarkable pressure. It is therefore difficult to believe, although its possibility is not doubted, that 15 mm. Hg represents an estimate of the thoracic duct pressure in the neck of a colt under ordinary conditions as reported by Weiss; and it is reasonable to consider, particularly in view of the lymph-pressures reported above, that an obstruction, possibly a clot, interfered with the flow through the cannula and caused the unusual lymph-pressure to be considered as normal.

Colin (2) reported in 1873 that lymph from the thoracic duct of a bull rose in a glass tube having a diameter of 5 mm. to a height of 108 cm.,

and later to 114 cm. He observed that a respiratory movement would cause the lymph to rise as much as 15 to 20 cm. at a time.

In 1920 Jappelli (5) reported his observations on lymph-pressures obtained by inserting a cannula directly into the thoracic duct of large dogs and attaching to this cannula a vertical glass tube 1.5 meters long. Lymph thus could mount in the tube and the various levels, indicating corresponding pressures, could be read off directly. He found that lymph under ordinary conditions rose to a height of 10 to 20 cm. in 30 seconds, and in one case (II) reached 60 cm., after one minute. Secondarily the lymph-level reached 70 and 110 cm., equivalent to 52 and 82 Hg respectively. He noticed also that the large fluctuations in the lymph-level were due to respiratory movements, and that the heart-beat caused small oscillations. Furthermore, he studied the effects on lymph-pressure of curare, section of the cord, and intravenous administration of salt solution in various strengths; but since these last mentioned factors have no bearing on the report they will not be discussed.

The dissection in the neck for the isolation of the thoracic duct as reported here follows in many particulars the directions for the establishment of a permanent thoracic duct fistula as recommended by Jappelli (4) in 1905; there are certain features, however, which it is believed are distinctive. In the first place it was found desirable, even necessary, to cut the left innominate vein in order to obtain good exposure of the posterior and inferior aspects of the venous angle; for frequently a small vein was found to follow along the first rib and enter the venous junction in such a manner that the vessel was not seen until the innominate vein had been severed. Furthermore, exposure was thereby gained to permit the ligation of tissues immediately beneath the venous angle, as well as to allow the ligation of the subclavian artery near the aorta. In the second place, in contradistinction to the recommendation of Jappelli, it was found more practicable to insert the lymph cannula into the external jugular vein not only because of its accessibility, but also because the valves at the terminus of this vein have been found to be further away from the venous junction and thus easier to reach than the valves at the mouth of the subclavian vein. This difference in the position of the valves held true for nine animals, and although it is not claimed that this relationship is a constant occurrence, its presence in nine animals appears more than a coincidence. In the third place, the insertion of a cannula in the subclavian vein was useful in furnishing a means by which the venous angle could be washed out as desired; for it is evident that Jappelli (5) was unable to observe lymph-pressure over a long time because of clotting in his apparatus.

The method of recording the extent of the lymph-pressure by the extent of rise in a vertical tube recalls the means adopted by the Rev.

Stephen Hales for measuring blood pressure in the horse; perhaps the only merit of this method is its simplicity. Yet a mercury manometer would not have been practical because of the small changes and the consequent difficulty of exact readings. A solution of sodium citrate could possibly have been better because of its anti-coagulating properties, but to employ it properly would have meant the frequent substitution of this solution for the lymph in the stem of the cannula, involving pressure changes, even though slight, at the entrance of the duct into the veins; furthermore, since clotting was not a difficult complication, not only because of the slow coagulation time of lymph, but also because of the wash-out system, the normal unmodified lymph served in these experiments as the manometric fluid. Only in one animal, LP6, immediately before death, was clotting not properly guarded against; but when the long record of LP5 is considered in which the readings extended for a period of almost two hours without clotting of the lymph, then the use of this fluid has some support.

The use of a cannula having a large internal diameter and a blunt end served to facilitate the passage of the fluid and also to free the apparatus of any constrictions which might affect the nature of the pressure change. Further, when it is remembered that the viscosity of lymph is 1.7 times the viscosity of water, then another argument for a large cannula is brought.

An obvious disadvantage of the method here described is the fact that no out-flow of lymph under the various pressures above zero is provided. Again, the proper size of the T-cannula needed consideration, for if it were too large then the rise of the lymph in the cannula would depend not only on the lymph-pressure but also on the amount of lymph, and if it were too small, capillary pressure and clotting would interfere. Jappelli did not mention the size of the cannula, but it must of necessity have been small; again the internal diameter of his manometric tube was not given, but from the rapid initial rise of the lymph called by him the primary rise, it is inferred that the diameter was small, and that the sudden rise at the beginning represented more a volume change than a pressure relationship. (See figs. 4 and 5.)

Another factor that must always be taken care of is the nature of the lymph obtained from such a dissection. If the lymph shows macroscopic evidence of blood, the observer is confronted with the problem of finding out whether the blood has gained entrance because of an incomplete isolation of the venous angle, or whether the blood has come up the thoracic duct with the lymph. It is not the purpose of this report to discuss the presence of blood elements, particularly red blood cells, in lymph, but it must be stated that all the animals from which the various curves and graphs for this report have been obtained did not show blood-stained

lymph. However it may be of interest to state that in one animal in which the same careful dissection was made, red blood cells were seen not only in the lymph coming from the thoracic duct but also in the lymph coming from the cervical lymph trunk; on the other hand, the lymph coming from the small intestine was clear. In another animal the lymph coming from the thoracic duct was apparently clear; but a post-mortem examination showed the presence of a small amount of blood in the lymph vessels leading from the bowel to the mesenteric lymph gland.

In the tracings, as shown in figures 1 to 3, the purpose was primarily to record relative differences, and no attempt, therefore, was made to determine absolute values for arterial pressure and lymph-pressure. From these tracings, on the one hand, the close relationship between lymph-pressure changes and intra-thoracic pressure variations, particularly with respect to respiration, was definitely recorded; but on the other hand, the tracings did not show the minute lymph-pressure changes associated with each heart beat which were visible to the naked eye, but which were nevertheless too small to be recorded with the manometer employed in these experiments.

An attempt was made to determine whether the small lymph-pressure changes because of the heart beat were due to intra-thoracic pressure changes associated with the contraction of the heart, or whether they were due to the pulsations of the subclavian artery transmitted directly to the overlying venous junction. To this end the subclavian artery was ligated near its origin; but nevertheless the oscillations of the lymph level occurring with the heart-beat persisted though possibly in less degree than before. No definite answer to the question can be given at this time, though the limited number of observations suggests that the phenomenon is to be accounted for through variations in intra-thoracic pressure, rather than to a transmitted pulsation from the thoracic aorta to the thoracic duct or from a pulsation of the subclavian artery. In this connection it may be stated that various light pressures on the thorax from without caused corresponding rises in lymph pressure.

However, a graphic idea of the successive measured increments of lymph pressure is gained from inspection of the curves shown in figures 4 to 7. Figure 4 represents a curve taken after the lymph had been allowed to drop freely from the cannula; it shows a curve of parabolic form with a gradual rise that came to the peak of 140 mm. lymph at the end of thirty-five minutes. From this peak the pressure receded a trifle for the next four minutes. This recession was probably due to a retrograde distention of the lymph system accompanied by a valvular insufficiency.

The readings for figure 5 were taken immediately after the lymph in the stem of the cannula as recorded in figure 4 was released, and the

tube clamped. As a result the type of curve is different at the beginning but subsequently takes on the general appearance of the previous curve. The quick rise in figure 5 is no doubt due to the fact that a certain amount of lymph had been held back because of the previous high pressure, and that this lymph when released rose comparatively rapidly in the tube. The peak of the pressure, however, did not quite reach the same height as that of the previous curve.

The curve shown in figure 6 is of interest in that it indicates some of the numberless ways in which lymph-pressure may be influenced. The beginning of the curve was very much like that shown in figure 4. At the end of thirty-five minutes the peak had presumably been reached, and the right hind leg of the animal was cleaned up preparatory to the injection of pilocarpin into the femoral vein. However the manipulations necessary for this procedure had excited increased respirations and the lymph-pressure accordingly rose. Subsequently shivering took place with a further rise in lymph-pressure, again due to respiratory forces, but possibly also to the increased flow of lymph known to occur because of muscular activity. The injection of pilocarpin caused the respirations to become more frequent and deeper, and at the same time the lymph-pressure rose to the height of 270 mm. Respirations ceased at this point and the lymph-pressure fell until a few spasmodic respirations again raised the pressure temporarily; however, from then on the pressure fell steadily until it reached 50 mm. at which level it remained. During the early part of this fall in pressure when respiration had ceased, the heart-beat was still accurately reflected on the lymph-level. Whether the rise in lymph-pressure following the administration of pilocarpin was due to the increased respiratory activity, or whether it was due to the contraction of the plain muscle in the viscera which forced the lymph forward, was not determined in these experiments. Obviously, a pneumo-thorax with artificial respiration would decide this as well as many other questions relative to the forces producing lymph-pressure. It is believed that the rise in lymph-pressure subsequent to the injection of pilocarpin was due to both factors, but mainly to the respiratory activities.

In view of the numerous valves in the thoracic duct it is difficult to understand the mechanism which allowed the lymph-level to recede gradually after respiration had ceased. One explanation would be that the amount of lymph which represented the fall from 270 to 50 mm., namely, 6.1 cc., was taken up into the large lymph trunks through incompetency of the valves and distensibility of the lymph vessel wall. However, the post-mortem examination did not reveal any extraordinary distention of the thoracic duct. A similar observation was made by Jappelli.

The curve in figure 7 represents the highest lymph-pressure recorded in this series, and assigning to the lymph an average specific gravity of 1.0165 (1), the pressure is equivalent to 355.7 mm. of water, or 26.1 mm. Hg. That this degree of lymph-pressure is not the maximum is shown alone by the rapidity with which it was reached, for if the labored breathing had continued for the same length of time in which the peak was reached under ordinary circumstances (about thirty minutes), a much higher value would have been obtained. Jappelli has recorded even a pressure of 82 mm. Hg. Certainly there are no forces producing an intra-thoracic pressure of 82 mm. Hg under ordinary conditions; and it is only the action of the accessory muscles of expiration, particularly the abdominal group, which could exert that much pressure.

The forces and factors responsible for the propulsion of lymph through its vessels in mammals have been described by physiologists as being: *1*, the small intrinsic pressure in the lymph capillaries derived indirectly from the blood pressure; *2*, the valves; *3*, the elasticity of the vessel walls; *4*, the contraction of muscle, whether it be the striated muscle of the extremities, the plain muscle of the intestines or lymph gland, or the heart muscle itself; *5*, ability of the individual inter-valvular segments to contract; *6*, the pressure changes in the chest associated with respiration; and *7*, the low or even negative pressure found in the venous angle. To these agencies may be added the peculiar arrangement of the entrance of the thoracic duct into the venous angle. The duct always enters in such a manner that its direction of flow makes an acute angle with the direction of flow of the venous channel into which it empties. Accordingly the lymph may be poured into the veins not only by virtue of the low pressure *per se* in the veins, but also because the rapid flow of the blood in the veins at their junction exerts a suction action on the current of lymph.

Of all these forces, it is believed that respiration is the most powerful. This is shown not only by the close relationship between intra-thoracic pressure and lymph-pressure but also by the experiment (fig. 6) in which the lymph-pressure fell immediately after respiration had ceased, and began to mount again when respirations were resumed for a time. Furthermore, in the case of the high lymph-pressure (fig. 7) the main force was probably due to respiration coupled with the powerful contraction of accessory muscles of expiration, particularly of the muscles in the abdominal wall.

It may be well to compare to some extent the observations of lymph-pressure as found previously with those reported here; but the comparison becomes difficult not only because the lymph-pressures were observed in different species of animals (cat, dog, colt, bull) and between animals

of the same species having marked differences in weight, but also because the pressures were measured in different lymph vessels (thoracic duct, cervical lymph trunk, tracheal lymph trunk). Comparing, however, the most recent observations (5) with those reported above, certain marked discrepancies are found to arise. In the first place the remarkable height of 110 cm. of lymph in the thoracic duct of the dog as observed by Jappelli, a height which is the same as found for the bull by Colin, differs radically from 15 cm. which was found under ordinary conditions in these experiments, and is still three times as much as observed when the animal was making labored respirations. Again the initial rise ranging from 10 to 30 cm. which was found to take place within 30 seconds was not observed in this investigation; on the contrary, even a casual inspection of figures 4 and 6 will show that the initial rise was not great nor rapid. It would be possible to reconcile these discrepancies if it were true that the internal diameter of the tube he used was 1 mm. or one-third of the one used here. But the size of the animals, the anesthetics, the dissections and the apparatus were so different in these two sets of experiments that a comparative study of their results is valueless.

Previous work by the author (6) has shown that following ligation of the thoracic duct in the chest in cats a collateral circulation is established to the right thoracic duct or to the azygos vein. It was also found that this collateral circulation developed at the end of seven days after the ligation. Knowing the time factor, the work here reported supplies, at least for dogs, the pressure factor, i.e., 11 mm. Hg.

Clinicians have speculated at great length on the possible effects of lymph obstruction, particularly in conditions of edema and elephantiasis. In two long articles Gross (3) has considered the increased pressure in the venous angle following valvular diseases of the heart as the cause for many edematous conditions. This contention does not receive much support from the observation that the pressure in the thoracic duct may easily rise to 11 mm. Hg in the dog, making it necessary to have a still greater pressure in the venous angle to cause a lymphatic obstruction; although the possibility, remote as it may be, that a persistent high venous pressure may gradually derange the forces of lymph propulsion and give rise to an edematous condition is not completely excluded.

SUMMARY

A method has been described by means of which lymph-pressures in the thoracic duct have been studied and kymographic records made.

It was found that the lymph-pressure in the thoracic duct reflected the intra-thoracic pressure, being highest at the end of expiration and depending on respiration mainly for its high values. Under ordinary conditions in the dog lymph rose by the end of a half hour to a height equiva-

lent to a pressure of 11 mm. Hg in a glass tube of 3 mm. internal diameter inserted into the venous angle in such a manner that the contents of the thoracic duct were directed solely into the tube; under conditions of forced respiration a pressure of 26 mm. Hg was recorded at the end of 16 minutes.

It is a pleasure to thank Dr. Harvey Cushing for his courtesy in extending the privileges of the Surgical Research Laboratory for this investigation.

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THE EFFECT OF INTRAVENOUSLY INJECTED SALINE SOLUTIONS ON THE VOLUME EXCRETION AND NITROGEN ELIMINATION BY THE KIDNEYS

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This work was begun to determine the rôle of saline transfusions in the amelioration of the symptoms of parathyroid tetany. Luckhardt and Rosenbloom (1) showed that completely thyro-parathyroidectomized dogs could be kept alive and relieved of all symptoms of tetany by frequent and large intravenous injections of Ringer's solution. It was moreover shown that calcium-free Ringer's solution was less efficacious in the improvement of the condition than was Ringer's solution, and it therefore seemed desirable to determine, if possible, the difference in action between the two solutions. The following explanations suggested themselves:

1. Ringer's solution may be more efficient because of a better physiological balance producing a more vigorous diuresis and a more rapid elimination of the nitrogenous bodies responsible for the tetanic condition.

2. The calcium present in Ringer's solution may combine with the toxins circulating in the blood and render them inert.

3. The calcium may alter the permeability of the cell membrane so that the toxins cannot enter.

4. The calcium after entrance into the cells, particularly of the nervous system, may render them less irritable.

5. A combination of all of the above four mechanisms may be responsible for the beneficial action of Ringer's solution.

Early in their work Luckhardt and Rosenbloom were impressed by the fact that water, containing 2.6 to 3.6 times the amount of calcium chloride present in Ringer's solution, when given *orally*, did not prevent the onset of tetany or control it when present. Luckhardt and Goldberg (2), in their work upon the oral administration of calcium lactate, made similar observations to show that water alone given by stomach tube will neither prevent nor control tetany. Such facts lead to the belief that calcium has some inherent physiological or pharmacological property as advanced in the second, third or fourth hypotheses above. But the obvious difficulties of testing these by our present physiological technic made it im-

perative to attack the problem from the diuresis side and either prove or disprove the first theory; namely, that by virtue of the calcium it contains, Ringer's solution is a better diuretic and produces a greater elimination of the protein-split products responsible for the tetany.

METHODS. Dogs, prepared for catheterization by a simple operation, were starved 24 hours and then given ½ pound of fresh cooked meat with a certain amount of water 4 hours before the experiment was begun. We found that under these conditions the animals had a fairly constant flow of urine at the beginning of the experiment, most of the water taken with the meal being eliminated within an hour before the experiment was begun. Exactly 3½ hours after feeding, the animals were placed on a table; an oiled silk catheter was introduced into the bladder, and the urine collected for the first half-hour was discarded. This procedure insured a quiet animal, the reflexes associated with the introduction of the catheter being eliminated. Urine was then collected continuously in 15-minute periods; after two such periods 25 cc. of saline solution per kilo body weight were injected intravenously. The injections were made with a Woodyatt pump, usually in 6 or 8 minutes. The collection of urine was continued for 3 hours at least, and the nitrogen content of the urine collected during each 15-minute period was determined by the Kjeldahl-Gunning method. Previous experiments had shown that too frequent catheterization traumatized the urethra and bladder so that traces of blood would occasionally appear in the urine. To remedy this, we did not use an animal without allowing at least one day to elapse between experiments. In spite of this precaution, and even with the greatest care in changing the position of the catheter in order to drain the bladder completely, small amounts of blood were passed in some of the experiments. Those urines that showed blood, either microscopically or macroscopically, were not analyzed for total nitrogen.

Ringer's, calcium-free Ringer's and 0.9 per cent sodium chloride solutions were injected in alternate experiments and, in addition, the normal rate of excretion without the injection of saline was determined. The Ringer's solutions used were identical in composition to those used by Luckhardt and Rosenbloom.

RESULTS. In this paper we will present only the results obtained from one animal. We have a complete series on this dog consisting of 6 experiments with Ringer's solution, 7 with calcium-free Ringer's solution, 5 with 0.9 per cent sodium chloride, and 3 control experiments. In order to simplify the data, the results of all the experiments are summarized in the following discussion of the four typical experiments given in table 1, which contains one each of the above experiments.

The initial rate of secretion is not uniform. Some of the experiments had an initial rate of 3 cc. per 15 minutes, others had a rate of 60 cc. for the same time. In general, the rate was between 4 and 10 cc. per period.

In the period during which the injection is made, and in the one immediately following, there is an increase in the flow of urine. The maximum flow is usually reached in the second period after the injection, and then drops abruptly toward the normal. This maximum varied, in one case reaching 81 cc., in another, 25 cc. The greatest number of experiments have a maximum flow of 40 to 50 cc. per 15 minutes.

About $2\frac{1}{2}$ hours after the injection there is a slight increase in the rate of flow in most cases, a secondary rise of short duration. This secondary

TABLE 1

EXPERIMENT 14		EXPERIMENT 5		EXPERIMENT 17		EXPERIMENT 20	
Vol.	N	Vol.	N	Vol.	N	Vol.	N
5.0 c	70.6	42.0	94.1	6.9 c	3.3	4.5	42.8
3.0 c	73.0	34.0	73.4	4.0 c	67.8	4.3	29.0
Injection of 400 cc. of							
Ringer's		Ca-Free R		0.9 per cent NaCl		Control	
40.0 t	81.6	33.0	69.9	38.9 c	12.4	3.0	53.6
81.0 c	353.2	55.0	44.0	51.5 c	109.2	3.5	56.4
16.0 c	94.7	20.0	66.4	25.0 c	164.0	4.0	46.6
12.0 c	50.9	6.0	49.2	11.0 c	100.3	3.0	50.0
6.0 c	61.0	5.5	00.7	11.0 c	45.3	3.5	64.0
4.0 c	50.8	7.0	67.5	19.5 c	152.1	3.5	39.0
9.0 t	110.1	10.0	60.8	28.5 c	90.1	2.5	11.2
8.0 t	127.0	8.0	56.6	21.0 c	4.2	3.0	120.0
6.0 c	97.7	10.0	62.4	17.5 c	2.8	2.5	105.5
7.8 c	89.3	13.5	59.4	21.5 c	145.3	3.3	108.0
12.0 t	147.8	10.0	72.0	6.0 c	144.0	2.5	111.5
8.0 t	79.7	7.0	50.9	8.0 t	118.4	3.0	111.2
7.0 t	61.0	5.0	24.4	6.5 t	51.5	2.5	81.5
		8.0	31.2				

Volumes in cubic centimeters.

Nitrogen in milligrams.

c indicates that the urine was clear; *t*, that it was turbid.

The character of the urines of experiment 5 were not recorded; in experiment 20 they were all clear.

rise is not of the same magnitude in every case; nor does it appear at the same interval of time following the injection. In some cases this secondary rise appears as soon as $1\frac{1}{2}$ hours after the injection; in others, as late as 3 hours. It reached a maximum in one case of 117.5 cc. in 15 minutes; in others it was just perceptible; while in 2 experiments the volume changes in succeeding periods were so slight that no secondary rise could be perceived. The secondary rise is not always of the same duration, sometimes persisting for $1\frac{1}{2}$ hours, sometimes for only one period (15 minutes).

The nitrogen content of the various urines is variable to a great degree. As seen from table 1 it may vary from 2.8 to 145.3 mgm. and from 0.7 to to 67.5 mgm., or it may remain fairly uniform, as shown in experiments XIV and XX. In most of the experiments the variations were irregular and of a marked degree.

TABLE 2

Averages of experiments that have uniform initial rates of secretion, and the percentage difference in diuresis

(Experiments 7, 8, 11, 14 used for Ringer's solution; 9, 12, 16, 18, 19 for calcium-free Ringer's solution; 10, 13, 17 for 0.9 per cent NaCl; and 15, 20, 21 for controls)

RINGER'S (4 EXPERIMENTS)	Ca-free (5 EXPERIMENTS)	0.9 PER CENT NaCl (3 EXPERIMENTS)	CONTROL (3 EXPERIMENTS)
6.12 cc.	4.94 cc.	5.30 cc.	6.63 cc.
3.81	4.20	4.16	7.55
4.96*	4.57*	4.73*	7.09
30.50	36.60	36.96	5.66
55.50	50.10	41.33	5.33
31.75	25.50	22.00	5.00
15.00	8.90	11.00	4.33
5.00	6.40	9.66	4.16
5.87	7.60	13.83	4.16
7.00	9.45	17.83	3.66
6.62	9.15	14.83	3.58
6.12	13.20	16.83	3.30
8.93	14.20	18.00	3.08
9.75	13.10	12.50	3.08
8.25	7.70	9.00	2.91
6.50	6.70	7.00	2.50
196.79	208.60	230.77	50.75
50.75	50.75	50.75	50.75
146.04	157.85	180.02	00.00
8 per cent		23 per cent	
Percentage of diuresis produced over Ringer's			

* Average rate of secretion in cubic centimeters during two 15 minute intervals prior to injection.

There is a variation in the physical character of the urines; most are clear, but some vary in *successive* periods from clear to cloudy or turbid, and then to clear again. Of importance is the fact that the nitrogen of a period is not greater with a cloudy or turbid urine, but is, on the contrary, often less. There is, however, no constant relationship between the physical character of the urine, volume output and its nitrogen content.

The rate of flow in the control experiments is uniformly less in succeeding periods, with slight variations. These slight variations are due, in all probability, to error in draining the bladder.

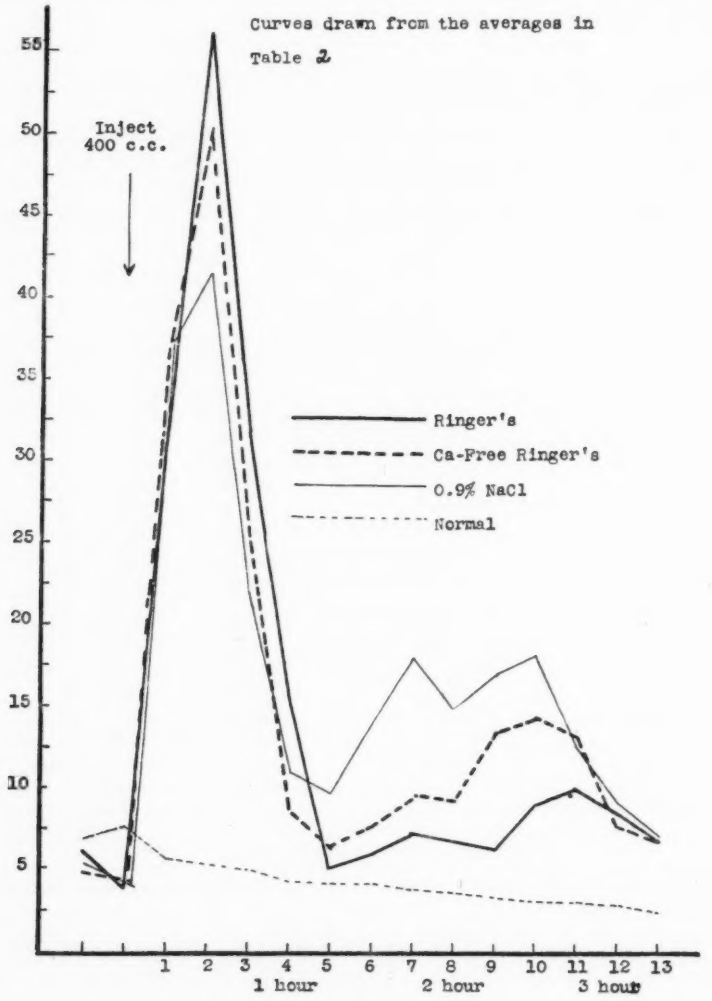

Fig. 1

I. Comparison of the solutions as diuretics. A consideration of above results brings out the fact that no definite data in regard to the

comparative value of the solutions as diuretics can be elicited from individual experiments. For this reason tables of averages were made, and as the initial rates of secretion vary greatly, and in order to make a comparison as exact as possible, the results of only those experiments that have a fairly comparable *initial* rate of secretion were used in the construction of table 2. From this table of averages it can be observed that the average initial flow is practically identical for each of the three diuretics, while the average initial flow is slightly higher in the case of control, and that the normal excretion rate decreases uniformly as the experiment proceeds. For the purpose of comparison, therefore, we assume that in each case, with a uniform rate of secretion, had no injection been made, the total amount of urine obtained would have been the same. In order to make a comparison of the diuresis alone, we subtracted the total of the average normal from the other totals. In spite of the fact that the average initial rate of secretion for the normal is slightly higher than the others, and in spite of the arbitrary calculations, we have a reasonably accurate means of comparing the diuresis alone produced by the three solutions, and have in this manner obtained the total percentage difference in diuresis. The curves, figure 1, plotted from the averages of table 2, show the following points, many of which have been brought out in the discussion of the individual experiments.

1. The primary rise is greatest with Ringer's solution, less with calcium-free Ringer's solution, and least with 0.9 per cent sodium chloride solution.

2. The rate of ascent and descent of the primary rise is practically identical for the three solutions.

3. The secondary rise comes on first with 0.9 per cent sodium chloride solution, and is of greatest height and duration. The secondary rise for calcium-free Ringer's solution is less marked; while the secondary rise for Ringer's solution is the least in height and duration, and is the last to appear.

4. The average volumes are almost identical in the last period.

5. In calculating the percentage difference in diuresis produced by the different solutions, we subtract the average normal flow from the average totals. It is a reasonable procedure, we believe, to subtract the volume that would have been excreted without the diuretic, in order to make a more accurate comparison. When the percentage differences are calculated, *it is found that calcium-free Ringer's solution produces 8 per cent more diuresis than Ringer's solution, and that 0.9 per cent sodium chloride solution produces 23 per cent more diuresis than Ringer's solution.*

These results receive a striking confirmation when the volume averages of table 3 are considered. This table was constructed for the purpose of comparing the nitrogen excretion obtained with these solutions, using all of the experiments in which nitrogen determinations were complete,

regardless of the initial rates of urine secretion. The result is, of course, a great diversity in the average initial rate of secretion. In order to make a comparison, therefore, we reduced the total average volumes to a mean in the following manner. We assumed that the normal rate of secretion of urine without an injection of saline is proportional to the initial rate of secretion; that is, if the normal with an average initial rate of 7.07 cc. will secrete a total of 47.55 cc. during the experiment, another series with a normal initial rate of 6.09 cc. should have had a correspondingly smaller total had no injection been made, for 7.07:47.55::6.09:X. The normal rates of secretion calculated in this manner were subtracted from the totals as was done in table 2 in order to make a comparison of the diuresis alone.

With the above crude calculations, the percentage differences in diuresis are even more marked than those obtained from the carefully selected experiments used in table 2. *Calcium-free Ringer's solution is found to produce 26 per cent more diuresis than Ringer's solution, and 0.9 per cent sodium chloride solution produces 68 per cent more diuresis than Ringer's solution.*

The fact that these experiments were conducted under uniform conditions, and the fact that only those experiments that had comparable initial rates of secretion were used in the construction of table 2 makes the result more striking. Even when calculated from diverse experiments selected with no regard for uniformity of initial rate, a similar difference not only appears, but is still more marked. As the experiments were alternated, it cannot be argued that variations in the condition of the animal were the cause of the differences in diuresis.

II. Comparison of the nitrogen excretion produced by the solutions. That no conclusions can be drawn from single experiments is more apparent when we come to a consideration of the nitrogen excretion produced by these diuretics. As already noted, the nitrogen excreted varies within wide limits in the same experiment. This extreme variation required that a large number of results be averaged. Accordingly, all the experiments with complete total nitrogen determinations were averaged in table 3, with their volume as noted above. Inasmuch as there is a diversity of initial rates of volume secretion and nitrogen excretion, no conclusions can be drawn from this phase of the work. But if the total nitrogen excretion be reduced to a mean in proportion to the average normal, some indication of the efficacy of these solutions in the promotion of increased nitrogen excretion might be derived. Following the same procedure as used for the calculation of the volume secretion outlined above, the nitrogen excretions were reduced to a mean, and the percentage differences calculated. The result shows that calcium-free Ringer's

TABLE 3

Averages of all experiments which have complete nitrogen determinations regardless of initial rates. Volumes in cubic centimeters, total nitrogen in milligrams

(Experiments 1, 4, 7, 11, 14, used for Ringers; 2, 5, 9, 16, 18, 19 for calcium-free Ringers; 3, 10, 13, 17, for 0.9 per cent NaCl; and 15, 20 for controls)

RINGER'S (5 EXPERIMENTS)		Ca-FREE RINGER'S (6 EXPERIMENTS)		0.9 PER CENT NaCl (4 EXPERIMENTS)		CONTROL (2 EXPERIMENTS)	
Vol.	N	Vol.	N	Vol.	N	Vol.	N
6.58	75.4	11.03	88.9	7.20	63.1	60.0	80.8
5.60	80.5	13.40	80.5	5.10	48.1	8.15	57.0
6.09	78.9	12.21	84.7	6.15	55.6	7.07	68.9
29.60	100.1	36.10	129.7	36.90	123.0	5.00	45.0
44.10	154.6	54.20	162.3	44.20	65.1	5.00	29.4
25.10	95.2	27.20	76.2	30.70	155.9	4.25	65.8
15.20	86.2	10.60	79.1	16.10	117.5	3.75	62.0
9.00	41.8	9.00	69.3	18.10	59.0	4.00	32.7
9.70	65.8	13.60	91.4	24.20	114.7	3.75	21.2
10.20	96.6	25.80	135.6	26.60	124.5	3.25	8.4
8.60	65.1	27.50	151.3	22.50	71.2	3.50	88.0
7.90	58.5	25.60	108.9	21.60	64.2	3.25	53.5
8.65	78.1	19.60	82.8	21.20	83.9	3.15	91.3
9.30	115.4	15.00	98.7	15.00	118.2	3.00	109.6
10.20	70.2	7.60	79.6	10.25	94.6	3.00	111.9
8.30	54.1	6.80	54.3	5.25	29.2	2.65	78.5
195.85	1081.7	278.60	1319.2	292.60	1121.0	47.55	797.0
40.75		82.60		41.77	Calculated normal		
155.10		196.00		250.83	Reduced to a mean		
26 per cent				68 per cent			
Percentage of diuresis produced over Ringer's							
Nitrogen totals							
1081.7		1319.2		1121.0		797.0	
912.8		978.3		642.2		797.0	
				Calculated normal			
168.9		340.9		478.8		000.0	
Nitrogen excretion reduced to a mean							
101 per cent				183 per cent			
Percentage of nitrogen excretion produced over Ringer's solution							

solution causes a 101 per cent greater excretion of nitrogen than Ringer's solution, and that 0.9 per cent sodium chloride solution causes a similar increase over Ringer's solution of 183 per cent.

The calculations for the nitrogen elimination are necessarily based upon crude assumptions. The results indicate, however, a confirmation of those obtained on volume secretion, namely, that contrary to our expectations, Ringer's solution is not only the poorest diuretic of the three, but also causes the least augmentation in the excretion of nitrogen. As no definite conclusions can be drawn from this phase of the work, the analysis of these solutions in relation to their ability to increase the nitrogenous elimination will have to be repeated and extended with more refined methods.

III. Variations in the physical character of the urine. As noted in the discussion of table 1, there are great variations in the nitrogen content and physical character of the urine in successive periods during the same experiment. The important features of these variations are:

1. A urine that has been flowing uniformly clear for 4 or 5 periods may, on analysis, show a variation in those periods from 10 to 300 mgm. nitrogen. This at first suggested an error in technic, but nothing was found in the method of analysis that could in any way account for the great variations.

2. A urine may alter in successive experiments from clear to cloudy or turbid, totally independent of the amount of flow, and as noted elsewhere, totally independent of the nitrogen content.

Discussion. By our method we have been able in a comparative way to study diuresis in the normal, unanesthetized dog. We find that the animals used are variable in their normal rates of excretion under constant conditions. The variations are most probably due to differences in the rate of digestion and metabolism after the uniform meal, and to the rate at which the water taken with the meal is allowed to pass from the stomach. This method of study, however, is far superior to methods that involve the use of anesthetics, and is, we believe, a refinement of the methods of Ginsberg (3) and of Cow (4), who also used unanesthetized animals. We find that dogs used in this work remain in good condition, and if reasonable care in catheterization is used, do not develop cystitis. There is no difficulty in draining the bladder completely; a slight movement of the catheter at the end of a collection period usually suffices to remove any residual urine. The objection to this method of collection as noted by Ginsberg is that blood is often passed with the urine. Cow in a later work (5) used a soft rubber catheter, which in our opinion is harder to introduce and to manipulate, and which, moreover, does not lessen the injury to the bladder.

The method, however, if carried over enough experiments, is valuable as a quantitative functional test of kidney activity.

Our results, especially in the case of the volume excretion, are very complex. The primary rise can be explained by the sudden increase in blood volume and the accompanying hydremic plethora. The diminution following the primary rise means that the unphysiological solution injected into the circulation has been largely taken from the blood and stored in the tissues. We believe that the following factors are operative in the production of the peculiar curves of diuresis:

1. The storage of water in the tissues. The work of Jappelli (6) shows that there is a storage of water in the muscle tissue in the case of saline injection. The osmotic pressure of muscle tissue is above that of blood, which means that the normal direction of water is largely toward the tissue, and that the normal direction of salt is from the tissue. That there is a considerable storage of water in our experiments is evident when it is considered that of the 400 cc. of solution put in, we rarely recovered more than 250 cc. in 3 hours. Japelli showed that the water storage is greatest with hypotonic solutions, and this is in accord with our findings.

2. The storage of salts in the tissues. Japelli further showed that there is a storage of salt that accompanies the water storage. With hypertonic solutions there is more salt taken up by the muscle tissue than with hypotonic solutions. With a solution such as the 0.9 per cent sodium chloride, which is in reality about 0.3 per cent hypertonic to blood in regard to sodium chloride content, we should expect a greater storage in the tissue than with the 0.7 per cent Ringer's solutions, and a consequent greater secondary diuresis when the salt is discharged.

3. The physiological action of the salts contained in the injected solutions. The work of Voit (7) in connection with sodium chloride and metabolism indicates that sodium chloride alone has an accelerating effect upon cell metabolism. If in connection with the above changes there is an increase in cell metabolism due to the sodium chloride content of the transfusion, then we should expect a secondary rise to be greatest with the solution containing the most salt; and as seen from the curves, the result agrees with the theory. But calcium-free Ringer's solution contains the same amount of sodium chloride as the normal Ringer's solution, and we find that it gives not only a higher secondary rise than Ringer's solution, but also 8 per cent more diuresis. This would indicate that either the calcium has a depressing effect, or that the loss of balance between it and the sodium and potassium leads to a more rapid elimination from the tissues in the case of a calcium-free solution.

Why there is a second diuresis is still unexplained. It must be remembered that the results of all the workers in this field, with the excep-

tion of Ginsberg and of Cow, were obtained from animals under anesthesia and as the above-mentioned workers used comparatively small injections, there is no secondary rise reported in their work. In a normal animal we cannot eliminate muscle tone, and it is possible that this factor may be operative, as suggested by Cushny (8), for in the anesthetized animal there is little or no return of lymph containing metabolic waste products, and this may be the factor in the secondary diuresis. Very often our animals shiver after a saline transfusion, and although this phenomenon is not always parallel with the secondary rise, it may have some significance.

That the final percentage differences in diuresis are due to the secondary rise for the most part is obvious, for an inspection of figure 1 reveals the fact that the differences in the primary rise are not great enough to cause the final diversity.

Therefore, as the solution that causes the most vigorous diuresis, 0.9 per cent sodium chloride, is the most unphysiological and contains no calcium, we can be safe in assuming that the factor of diuresis is not of prime importance in the prevention and control of tetany by these solutions. Luckhardt and Rosenbloom state that they were able to keep dogs alive with calcium-free Ringer's solution. But those dogs, with one exception, that were kept in good condition with calcium-free solution, were well past the danger point (9). In view of the work of Luckhardt and Goldberg on calcium lactate by mouth, the argument presented here, that diuresis is not a deciding factor in the control of tetany, is further strengthened. On the basis of this work, and on the basis of the work of Luckhardt and Goldberg, it must be assumed that calcium given intravenously in the form of Ringer's solution must have some inherent physiological property which is operative in the control of tetany. That intravenous injections of pure and concentrated calcium solutions will not preserve the life of an animal, is indicative of a complex situation in which the exceedingly unphysiological means of administration and the resulting damage, may entirely overbalance any beneficial action.

The work in connection with nitrogen excretion must be regarded wholly as preliminary for there are not enough results to lead to definite conclusions. But if the indications are correct, the best elimination of nitrogen is obtained with the least physiological of the solutions, the 0.9 per cent sodium chloride; which is further evidence to support the view that calcium is not solely operative in the removal of toxins, but must have some other more important function, possibly in the neutralization of toxic nitrogenous compounds. The complication of the work on nitrogen by the uncontrollable factors of metabolism and digestion will in further work be eliminated by the use of animals in a post-absorptive condition.

The extreme variability of the urine in regard to nitrogen content and physical character is an observation of importance in relation to the physiology of metabolism and excretion. For these variations come at the

height of digestion, 5 to 8 hours after the meal, as determined by Voit, Feder, Gruber (10), and McEllroy and Pollack (11), and from our studies of them in connection with other work, we are led to the conclusion that the kidney functions in a manner more complex than hitherto imagined.

By means of this method of study of urine secretion, we have been able to demonstrate that the administration of calcium lactate intravenously in an attempt to control tetany is extremely unphysiological. This work, which is now in progress, has so far shown that the intravenous injection of calcium lactate exerts a deleterious effect upon the organism in general, and on the kidney in particular, in that it has a mild nephropathic action and will, even in a normal animal, produce a transient anuria and albuminuria, the extent of which depends upon the dose.

SUMMARY

1. A method for the collection of urine and the study of diuresis in the normal unanesthetized dog is described. This method is applicable as an experimental functional kidney test.

2. A study made of the efficiency of Ringer's, calcium-free Ringer's, and 0.9 per cent sodium chloride solutions as diuretics shows that the former is the poorest and the latter the best, in comparison.

3. A peculiar, inconstant phenomenon, the secondary rise in diuresis, greatest with 0.9 per cent sodium chloride solution is described.

4. Sudden variations in the nitrogen content and the physical character of the urine collected at the height of metabolism are described.

5. On the basis of this work, and on the basis of the work of Luckhardt and Goldberg, it can be assumed that calcium has some inherent physiological or pharmacological property that operates in the amelioration of the symptoms of parathyroid tetany, and that the diuresis produced by the injection of Ringer's solution is not the factor of prime importance in the improvement of the condition.

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ON THE PENETRATION OF ACID AND ALKALI INTO LIVING CELLS AND ON A PROTECTIVE MECHANISM OPERATIVE IN CULTURES OF AMOEBOCYTE TISSUE[1]

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In previous investigations, in using the tissue culture method, we obtained from experimental amoebocyte tissue the formation of layers of tissue as the result of the migration of amoebocytes into the culture medium not only when neutral solutions of various osmotic pressures, but also when decidedly acid or alkaline solutions served as the culture medium. In certain cases we found that addition of acid and alkali not only allowed the outgrowth to occur, but that it even increased it (1), (2), (3). Two problems arose in this connection: 1. If the outgrowth occurs in an acid or alkaline medium, do the acid and alkali penetrate into the cells, or do these substances produce their effects by acting merely on the cell surface? 2. Do the salt solutions, into which the cells move so readily, serve as a satisfactory culture medium for the cells, or is some protective mechanism at work which somehow modifies the effect of these solutions?

As to the action of acid and alkali on the cells, we considered both the possibility that they act merely on the surface of the cell, as well as that they exert their specific effects after penetration into the cells, without however being able to decide this question definitely. In particular the changes brought about, by the application of the acid, in the character of the cytoplasm suggested the possibility that acid and alkali might actually penetrate into the living cells (3), (4). Opposed to such a conclusion were the observations especially of Warburg, Harvey (5), Jacobs (6), (7) and others, which proved that the fat soluble carbon dioxide and ammonium ions penetrate readily into living cells, in contradistinction to the ordinary inorganic acids and alkalies. On the other hand, in the case of an alga, M. M. Brooks (8) has quite recently shown that the ordinary inorganic acids penetrate into the living alga much more readily than had been assumed.

In order to understand the action of acids and alkalies on cells in tissue cultures, it was necessary to determine the mode of attack of these substances on cells. As to the action of neutral salts on cells in the tissue

[1] As to the terms used in this paper, we refer to our previous publications on this subject.

cultures, we had previously suggested the possibility that substances derived from the tissues as well as serum might become admixed to the solution of these salts and might thus modify their effects on the cells and make them suitable as a culture medium.

We hoped to obtain a more definite answer to these questions by modifying our mode of experimentation; instead of studying the outgrowth of the tissue into the various culture media, as we had done previously, we allowed first an outgrowth of tissue to develop in the usual manner and then studied the effect of the addition of various solutions directly to the sheet of the tissue. For this purpose we drew off the fluid covering the cells with strips of filter paper and replaced it by solutions, the effect of which we desired to investigate. In this way it was possible, repeating if necessary this process several times in succession, to observe directly the action of various substances on the tissues.

In our experiments we used specimens of tissue which had grown out into various solutions of sodium chloride, as well as into Limulus serum. In some cases sheets of tissues, which had developed in acid and alkaline solutions of sodium chloride, were used. These tissues were kept for different periods of time, varying from one to about seven days, in the ice chest, before they served for these experiments. The following series of experiments was carried out:

1. Addition of neutral red and n/2 NaCl solution. A small amount of a (1:4000) solution of neutral red in a n/2 solution of NaCl is added to the tissue. After a lapse of from one to five minutes the staining solution is removed and replaced by a n/2 solution of sodium chloride which is almost isotonic with the blood serum of Limulus. Within a very short time, usually within the first minute, the cells stain diffusely with neutral red. The stain is localized in the granules which normally fill the cell. In addition we notice after some time that the stain collects in some particles or drops in the interior of the cell. In cells which have lost their granules and have become hyaline, the stain is limited to these particles or drops. There may be several of these drops, or there may be present merely one large drop of stained fluid in the cell. Gradually in a granular cell the stain is given off by the granules more and more and in the end the stain is found only in the particles or drops. In these drops or particles the stain may remain for a considerable period of time. The rapidity with which the stain disappears from the granules varies in different cases. In some cells there may still be a diffuse stain of the granules on the following day.

The granules take on a red brown color with neutral red, but the intensity with which the granules stain depends somewhat on the previous treatment of the tissue. Cells which have grown out in Limulus serum stain usually deeper than cells which have grown out in solutions

of sodium chloride. As we shall see later, an alkaline reaction of the surrounding medium likewise makes the stain of the granules more intense.

If we use for our experiment, tissue which has been growing for one or several days in a solution of n/2 NaCl, or in serum, and in which the cells have undergone the usual changes and have moved out of the piece of tissue with tongue pseudopodia, we find after replacement of the neutral red solution by a few drops of a n/2 NaCl solution a very rapid change in the character of the cells. Some cells may contract at first, but within a minute or two, or somewhat later, drops and balloons appear in the cells, especially in the peripheral ones, while in the more central cells tongue pseudopodia may still predominate. These drops or balloons may develop in a tongue pseudopod or elsewhere in the cell; occasionally a tongue pseudopod may become transformed into a series of drops, arranged one after the other in a straight line. The granuloplasm moves into these balloons and drops connectedly and through this process the latter are filled out by granuloplasm and thus cells may be produced, the outline of which somewhat resembles mulberries. After a balloon has in this manner undergone a fate similar to that of a tongue pseudopod in a normal cell, a new balloon or drop may be sent out. Such balloons or drops may be temporarily polarized and then the cell as a whole is able to change its location through these movements of the granuloplasm into the drops or balloons. In other cases no polarization of balloons or drops occurs, and this is especially true when multiple drops have been formed.

Subsequently this process may extend still further: the number of balloons usually increases in the course of the next few hours, and structures may develop which we designated formerly as courts and others which resemble fertilization membranes of ova. Occasionally, but on the whole not very often, circus movements and pari passu movement[2] occur under those conditions. These changes take place especially in contracted cells, while the cells which have extended have the tendency to hyalinize or dissolve.

It seems that the character of the solution in which the tissue has grown out has some influence on the end result. Thus cells which have previously been in serum seem to be more reactive and to form balloons more profusely than tissue which has grown out in a n/2 NaCl solution. It seems furthermore that tissue which has previously been in a hypotonic solution, as for instance n/4 NaCl, has a greater tendency to form tongue pseudopodia rather than balloons, while, on the contrary, tissues which have previously been kept in hypertonic solutions like those of 11/16 n

[2] During pari passu movement the balloon formation and the movement of the granuloplasm into the balloon take place simultaneously.

NaCl, seem to have a greater tendency to develop balloons after application of a n/2 NaCl solution. However, it will be necessary to determine the influence of the preceding solutions in further experiments.

We see then that the substituton of the original sodium chloride solution, in which the cells have grown out, by a new solution of sodium chloride, identical in compositon with the one originally used, produces effects on the cells which are different from those produced by the original. solution. Evidently the change of the solution leads to the entrance of fluid from the surrounding medium into the cells and this results in the production of drops, balloons and structures, resembling eggs with fertilization membranes, and in processes of solution in the extended cells. We must assume that the pouring on of the second solution of sodium chloride leads to the removal of a mechanism which protected the tissue originally growing out into the sodium chloride solution, against some of the injurious effects of this salt. However, even in tissues, which in the usual manner grow out into a n/2 NaCl solution, ultimately the formation of drops may be observed especially in the peripheral cells. The change of the fluid thus accelerates and intensifies changes in cells which would take place otherwise much more slowly.

2. Addition of a n/2 NaCl solution without neutral red. If we replace the original culture medium by a new solution of n/2 NaCl without a previous addition of neutral red, the effect is somewhat similar but probably less marked. The number of balloons and drops seems to be smaller, but some drops or balloons usually form in the periphery. Rarely pseudofertilization membranes and courts originate under those conditions. Granuloplasm moves subsequently into the drops and balloons which thus disappear. The vacuolization and solution of the extended cells is much hastened through the change in the fluid and the loss of granules and the hyalinization of the contracted cells which is common under certain conditions may likewise be accelerated. All these effects are more marked if the solution of n/2 NaCl is changed repeatedly instead of once only. We may then conclude that this change of fluid accelerates those processes in the cells which would otherwise take place much more slowly, and we may furthermore conclude that the addition of neutral red increases in all probability the effects caused by the change of fluid. The increase in the injuriousness of neutral red for cells, when exposed to light after they have been stained, plays apparently no rôle in this case. Previously we have shown that exposure to light makes solutions of neutral red injurious to eggs (9).

3. Addition of Limulus serum. If a tissue has grown out into a solution of n/2 NaCl and this fluid is substituted by Limulus serum, the outgrown layer, which had previously the character of a tissue grown in an isotonic sodium chloride solution, assumes now more or less the charac-

ter of serum tissue: the previously contracted cells spread out within a very short time, the pseudopods become broader and may even change into balloons; and occasionally circus movement may occur in such cells. Granuloplasm enters as usual the balloons and broad pseudopods. The extended cells dissolve and mere shells of cells may remain. Previous addition of an isotonic solution of neutral red to the tissue causes the cell granules to stain red brown; and again the stain disappears gradually from the granules. It may perhaps increase the number of balloons and courts and pseudofertilization membranes which appear under those conditions. On the whole then the addition of serum accelerates the changes otherwise brought about more slowly by serum into which the cells moved originally; but as compared to solutions of sodium chloride, serum seems to have a relatively preserving effect upon the tissue. The number of balloons and drops which form is smaller and the solution of granules is less marked in serum than in isotonic sodium chloride. The spreading out of the cells and the broadening of the pseudopods are characteristic of serum tissue.

4. Addition of hypotonic solutions of sodium chloride. Solutions of n/4 or n/3 sodium chloride, when added to tissue which has developed in isotonic solutions of sodium chloride or in serum, have effects characteristic of a hypotonic medium. Within a few minutes they cause the rapid formation of very large, often multiple balloons; also of courts and pseudofertilization membranes. Small drops are usually absent. When the granuloplasm enters the balloons, which may occur rather rapidly, mulberry-like appearances may be produced. Under these conditions the cells take up fluid rapidly, and thus the extended cells dissolve or hyalinize quickly; in the central parts cells are especially prone to hyalinize. The effect of the hypotonic, in contrast to the isotonic solution, consists then in the larger size and the more rapid appearance of the balloons and of similar structures, in the relatively smaller number of tongue pseudopodia and in the wider extension of these changes in the direction toward the center of the tissue. As early as one to two minutes after addition of the fluid the balloons may appear even in the central cells. The solution of extended cells also takes place rapidly and this may represent the first change that occurs; at a later stage the texture of the cells is generally found to be loose owing to the imbibition of the hyaloplasm with fluid; the granules are also somewhat swollen and a number of granules are dissolved. In hypotonic solutions these changes take place even without the previous addition of neutral red.

5. Addition of hypertonic solutions. If, instead of using hypotonic solutions of sodium chloride, we use under the same conditions solutions of 11/16 n NaCl, hypertonic changes are produced resembling those observed in cells primarily growing out into a 11/16 n NaCl solution, but

somewhat modified by the change in solution. The cells contract somewhat, become relatively small, and instead of sending out large balloons, they produce small droplets or they form sharp tongue or thread pseudopods; droplets or balloons may change into tongue pseudopods or broad tongue pseudopods may become thinner and develop long threads. Sometimes these changes become more noticeable only after a second addition of 11/16 n NaCl, and only then balloons may change into tongue pseudopods. Repeated changes of 11/16 n NaCl may also cause hyalinization of the cells. Granuloplasm usually enters the droplets and mulberries are produced. As early as within five minutes after addition of the solution a hypertonic field may thus be produced which is typical except that the cells are rounder than is usual in 11/16 n NaCl. Under these conditions water is withdrawn from the cells and the consistency of the protoplasm increases.

6. Addition of acid followed by alkali. In a series of experiments we tested the effects of acid and alkali on the sheets of tissue which had previously developed in neutral solutions. First we added an isotonic solution of neutral red; after the stain had been allowed to act on the tissue for from one to five minutes, the supernatant fluid was drained off and replaced by a solution of n/1000 HCl in n/2 NaCl; in some cases this solution was renewed once or several times. After the acid had had a chance to act on the tissue for variable periods of time, it was replaced by n/1000 NaOH in n/2 NaCl.

The addition of acid causes a rapid loss of the neutral red stain especially in the peripheral cells. This occurs usually within the first minute following the pouring on of the acid. Sometimes several washings with acid have to be used until the decolorization has become complete. After the granules have been decolorized, some pink droplets or particles of neutral red may remain visible in the cells for some time. Very soon acid causes a contraction of some cells and the formation usually of a limited number of balloons or drops especially in the peripheral cells. This is followed by a movement of the granuloplasm into the balloons or into the multiple drops. The tongue and thread pseudopodia are gradually withdrawn and the remaining ones are filled by granuloplasm. Thus mulberry cells are formed, which subsequently round off. Inasmuch as new balloons or drops do not usually form, a state of rest is gradually produced in this acid solution. The acid which penetrated into the cells decolorized and paralyzed them, without however killing them.

If, after withdrawal of the acid, we now add a solution of n/1000 NaOH in n/2 NaCl, the cells are revivified. Within a minute or two the peripheral cells become stained again with neutral red. They do not usually regain their full coloration, the staining remaining weaker than it was originally. If we wash off the alkali with isotonic salt solution, add then

acid, these cells lose their stain a second time, and if we now add alkali once more the peripheral cells may regain some of the neutral stain; but the intensity of the stain has progressively diminished. If previous to adding the alkali, we remove the central piece of tissue from which the outgrowth of the cells took place, the coloration, which the cells are able to regain, is still less.

These observations make it very probable that the remnant of neutral red, which has been left in the original tissue as well as that in the fluid, is the source which provides the stain which after the addition of the alkali penetrates again into the cells. The oftener the surrounding medium is changed, the smaller the quantity of fluid which is left; and by removing the central piece of tissue this depot of neutral red is likewise removed.

Soon after the addition of the alkali some drops and balloons are usually produced in the peripheral cells. Again they are filled out by granuloplasm or are retracted. In typical cases there follows a second stage of the alkali action in which tongue and thread pseudopods are formed, especially in the periphery of the sheets of tissue. Frequently the large majority of the cells may undergo this change but in other cases the number of cells in which these pseudopods develop is more limited. As in other cases drops or balloons become sometimes converted into tongue pseudopods, or threads may develop on top of the balloons or drops. Occasionally some drops or balloons which had formed in the first stage may persist in this second stage. The time when this stage begins varies somewhat; it may occur as early as within a few minutes, or it may occur in ten minutes, or still later, after addition of the alkali. This second stage is almost always followed by a third one in which again drops and balloons develop; and gradually the latter increase more and more in number. As usual the peripheral cells are first affected and here more pronounced changes take place.

From this typical sequence of events some deviations may occur. Thus in alkali the stage of tongue and thread pseudopods may be minimized, or, it may occasionally perhaps be lacking altogether; while on the other hand in acid some tongue and drop pseudopods may persist through a longer period of time.

We interpret these reactions as indicating a rapid penetration of acid and alkali into the amoebocytes. In the original neutral solution the stain is able to penetrate into the cells and to combine with a constituent of the cell granules. In the amoebocytes the reaction is apparently very slightly alkaline to judge from the red brown color of the stained granules. As soon as a very weak isotonic solution of HCl is added, the latter penetrates into the interior of the cells, leading here to the production of an acid reaction and thus to a rapid decolorization of the granules.

At the same time the addition of the acid causes the production of balloons and drops and allows the movement of the granuloplasm into the tongue,—drop,—and balloon pseudopods to take place. This is followed by a state of contraction and rest in which further amoeboid activities are suspended. If alkali is now added, it penetrates likewise into the interior of the cells and leads to a restaining of the granules, provided a sufficient amount of dye is still available for this purpose.

The interpretation of the various phases of alkali action which we observed can only be a tentative one at the present time; but we may assume that the change of the fluid as such acts as a strong stimulus to which some of the peripheral cells respond with the production of balloons and drops. This is followed by the penetration of the alkali into the interior of the cells, and a gradual neutralization and subsequent alkalinization of the cell. At a certain intermediate stage of this process sharp tongue and thread pseudopodia are sent out. Gradually the alkalinization proceeds further and when a certain stage in this process has been reached, the proteids of the cell attract a larger quantity of water, and the formerly sharp pseudopods became broader, drops and balloons form and the extended cells are dissolved. We might assume that the stage of the formation of sharp pseudopods corresponds to the approximate neutralization of the acid; but against this interpretation might be cited the fact that addition of neutral isotonic sodium chloride solution leads to the formation of balloons and not of tongue pseudopods. We have therefore to consider the possibility that this stage corresponds to a condition when the state of the cell is still very slightly acid or alkaline.

7. *Addition of alkali followed by acid.* If, after pouring on of the isotonic neutral red solution, alkali is added to the fluid, the staining of the cell granules is intensified; they assume a deeply red brown color. Otherwise the addition of n/1000 NaOH in n/2 NaCl has the same effects as the addition of a n/2 NaCl solution, but the effects are probably more marked, if alkali is used. The cells round off, balloons, multiple drops, pseudofertilization membranes and courts are formed and, in many cases, the extended cells dissolve. While some tongue pseudopodia develop, perhaps, they are not prominent under those conditions and the typical sequence of events which we find, when alkali follows the addition of acid, is lacking in this case. This is perhaps due to the absence of the neutralization process following the primary addition of alkali. If now secondarily acid is added, the acid penetrates again into the cells, causes a disappearance of the stain and, after a temporary formation of drops and balloons and perhaps the passing appearance of some tongue pseudopodia, the granuloplasm fills out these formations, the cells round off and come to rest. After a second addition of alkali some cells may restain

and a number of cells may resume their activity, forming balloons or tongue pseudopodia.

If a stronger solution of alkali (n/100 NaOH in n/2 NaCl) is added, the effect is much more pronounced. The cells round off and form balloons and perhaps also pseudofertilization membranes; then the taking up of water by the cell proteins becomes still more marked and many cells burst, scattering their cell granules into the surrounding fluid. If, on the other hand, a piece of tissue is placed on a cover glass and surrounded by a n/100 solution in n/2 NaCl, the majority of the cells which move out into this fluid remain well preserved for a considerable period of time. Under these conditions a protective mechanism is present which is apparently destroyed when the solution is secondarily directly poured on the layer of outgrown cells.

Conclusions. 1. In cultures of amoebocyte tissue a mechanism exists which protects the cells which migrated into the surrounding medium against certain injurious effects of this medium. As we have previously shown the character of the medium employed has a definite effect on the outgrowing layers of tissue. These effects are not only qualitatively dependent on the character of the solution, on the kind of ions, the osmotic pressure, the hydrogen ion concentration used, but there is also a quantitatively graded relation between these factors and their effects on the cells, and in particular on the consistency of the protoplasm which later seems to be a determining factor in the amoeboid movement.

The solutions have the most marked effect in the periphery of the area of outgrowth and their effect often becomes less near the central piece of tissue.

These experiments prove that the effects of an isotonic sodium chloride solution on the amoebocytes are much stronger, if added to the layers of outgrown tissue, than if the cells are allowed to migrate into this solution from the original piece of amoebocyte tissue.

This protective influence which is effective in the latter case resides evidently in the protein material which is produced in the culture medium. While this protein material may be derived partly from blood serum admixed to the tissue, it originates largely through the dissolution of emigrating cells and of the margin of the transplanted piece which takes place in different cases to a varying degree, according to the character of the solution used. This protein forms a protective layer which is densest near the piece, and in certain cases we can observe with the naked eye such a protective layer covering the cells, especially in the central part of the specimens. Thus, as stated above, we may explain the fact that the effects of the solution are usually most marked in the periphery Here the vital stains penetrate first into the cells and here also pathological

effects of the solutions are likely to appear earliest. At least this applies as far as the contracted cells are concerned; the extended cells, on the other hand, are more readily accessible to the injurious and dissolving action of the solutions, and inasmuch as extended cells may be found in a considerable number also in the more central parts of the tissue layers, this dissolving effect of the solution may be quite marked at the latter place as well.

If the cells are allowed to move slowly into the culture fluid such a protective envelope can function, but if the fluid is withdrawn and new fluid is poured on, and especially if this process is repeated several times, this protective envelope is liable to be disturbed even if the changing of the fluid is carried out as gently as possible, and thus the pure solution can act directly on the cells. It produces rapidly over a wide area effects which, if the ordinary procedure is applied, occur much more slowly and are usually limited to the periphery. These effects can all be referred to the entrance of fluid into the cells, and to a subsequent decrease in the consistency of the protoplasm, with the formation of broad tongue-, balloon- and drop-pseudopodia, hyalinization processes and solution of extended cells. The cells cannot successfully resist the entrance of the fluid under those conditions.

While these effects are more or less common to all the solutions used, there are in addition noticeable specific effects characteristic of each solution. Thus serum of Limulus, hypotonic, isotonic and hypertonic solutions of sodium chloride, all exert their specific effects and usually within a very short time after they have begun to act on the tissue. These specific effects are in accordance with those which we obtained previously in using the ordinary tissue culture method.

2. Our experiments prove that weak and isotonic solutions of hydrochloric acid and sodium hydrate may penetrate very rapidly into the living amoebocytes and that they can here produce their typical effects without killing them. Acid produced contraction and, following transitory amoeboid changes, a state of inactivity; while alkali called forth definite amoeboid activity. Under certain conditions the application of alkali produces the definite sequence in the amoeboid activities, which we described above.

While it is thus certain that the effects of acid and alkali on amoebocyte tissue, which we observed previously (3), are dependent not merely on membrane changes but on intracellular action of these substances, yet it is very probable that the same protective effect, which we analyzed above, comes into play also under these conditions and that the acid and alkali, acting in a quantitatively graded manner on the tissue, are weakened in their effects through the proteid envelope of the tissue. The effect of this envelope may be mechanical as well as chemical in char-

acter. A part of the acid or alkali combines with the proteid and becomes inactive, as far as the cells are concerned. Thus it is brought about that gradually the concentration of the acid and alkali used becomes weaker and the reactions of the tissue change accordingly.

3. We may assume that whenever substances exert an effect within the body, they act on the tissues and cells under conditions somewhat comparable to those prevailing in a tissue culture, which we have analyzed in these experiments. We may therefore assume that protective mechanisms of a similar nature to the proteid envelopes exist also in the body.

With these observations agrees the fact that Limulus serum does not act on the amoebocyte tissue merely like a combination of inorganic salts, but that its proteids exert a definite protective effect. As we have shown here protective proteid envelopes of a somewhat different character act on cells also in various solutions of salts and acids.

4. We have seen that the entrance of acid into the cell causes the almost instantaneous disappearance of neutral red stain from the granules. It is most probable that under the influence of acid the neutral red base is dissociated from the granules and passes out of the cells.

We have furthermore seen that if the amoebocyte is stained with neutral red in a neutral solution the granules gradually give off the stain. This takes place, not only if we apply a neutral solution, but even if we apply a slightly alkaline medium, in which the cells move after having been stained. In this case also the stain disappears gradually from many granules, although apparently not as rapidly as in a neutral solution. However, in the experiments in which we gave special attention to the staining or decolorization of the granules after application of neutral red, the hydrogen ion concentration happened to return almost to the neutral point at the end of the experiment. Without denying the possibility that reduction processes may be responsible for the disappearance of neutral red we may in addition suggest that at least one of the factors which may cause the decolorization on the part of the granules may be a localized and passing production of acid within the cell. We have every reason to assume that acids are produced temporarily during the metabolism of motile cells and are soon destroyed. Under certain conditions therefore the disappearance of neutral red stain from the granules may be an indication of acid production within the cells. However, at present we are not justified in assuming that this is the only cause of the disappearance of neutral red from the granules. In this connection we might cite an observation of Chambers, according to which in egg cells also the neutral red disappears spontaneously from the granules (10).

When the stain has disappeared from the granules, it may still persist in certain drop-like structures which are usually found under these conditions in amoebocytes and which we mentioned above. But while the

granules always seem to assume a red brown color, indicating a slightly alkaline reaction within the cell, these drop-like structures may assume a distinctly pink color, especially in an acid medium. The acid evidently does not cause the elimination of the neutral red from these latter structures in contrast to the granules which lose their stain in an acid medium.

5. The observations here recorded confirm again the connection between processes apparently so different in character as the formation of fertilization membranes in ova, on the one hand, and amoeboid movement in motile cells on the other. In both cases we find in response to a stimulus the raising of a cell membrane by proteid material of cellular origin which is much diluted by fluid which is at least partially derived from the outside. We can designate the production of a fertilization membrane as the sending out of a generalized pseudopodium, or conversely, we may designate the pseudopod as a localized formation of a fertilization membrane. The amoeboid cell differs from others mainly in the processes which follow the raising of the membrane, localized or general; but even in this respect the differences are not so marked as is usually assumed. The varieties of cells which under certain conditions are capable of amoeboid movement is very great and includes cells which usually behave as fixed.

This applies even to egg cells in which, according to observations of M. H. Jacobs,[3] through a moderate exposure to heat it is possible to cause the separation of a clear exoplasm from the granuloplasm in pseudopod-like processes.

The production of drop-like formations on the surface of Infusoria, which has been observed under certain conditions, is probably a related process, although amoeboid movements do not occur under these conditions.

SUMMARY

1. When we compare the effect of solutions serving as culture media for amoebocyte tissue with the effect of the same kinds of solutions, when added to the outgrown layers of cells, we find in the latter case the action in principle similar, but quantitatively much stronger and there is noticeable a marked accentuation of the pathological end effects of these solutions. For this and other reasons we must assume that in cultures of this tissue (as presumably also in the living organism) protective mechanisms exist, consisting in envelopes of proteid material, which surround the cells and mitigate very much the effects of substances which are added from the outside, without, however, abolishing these effects.

[3] Personal communication.

2. The ordinary inorganic acids and alkalies penetrate very readily into the amoebocytes and thus exert their typical effects. In this case again we have reason to assume that in the tissue culture (and in the living organism) proteid envelopes exert a protective action.

3. Acid causes the rapid decolorization of the cell granules. Such a decolorization occurs also spontaneously in neutral or even in slightly alkaline solutions, although much more slowly and gradually under those conditions. A transient formation of acid within the cell may perhaps be one of the causes of the spontaneous loss of neutral red stain on the part of the granules.

4. The observations here recorded add further evidence pointing to the relationship between the processes underlying amoeboid movement and the formation of a fertilization membrane. A pseudopod can be conceived of as a localized fertilization membrane and a fertilization membrane as a generalized pseudopod.

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A RELATIONSHIP OF BLOOD SUGAR TO THYROID AND SUPRARENAL SIZE IN A FRATERNITY OF PIGEONS

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In connection with earlier studies made by the senior authors on the blood sugar of more than 700 doves and pigeons the necropsies of most of the animals were soon obtained in order to learn whether they were healthy or diseased. Other work simultaneously carried on at this laboratory required the routine dissection and weighing of several incretory and other organs of all birds thus killed. In addition to these measurements of size of organs very little histological work could be undertaken; and some organs, such as the pancreas, could not be advantageously included in these studies. It was thought nevertheless that in a study of so large a number of individuals it might be possible to establish a relationship between the blood sugar on the one hand and the size of some incretory organs on the other. The great bulk of our material has proved quite disappointing in these respects, but within a single large fraternity (brothers and sisters) of dove hydrids we are able to show that this type of relationship does exist in the case of thyroids and suprarenals. The relationship obtained is not of the type anticipated by us, and we find ourselves unable fully or satisfactorily to interpret the results obtained. The relationship observed, however, is doubtless a fact of some importance, and since similar data are unknown it seems advisable to put them on record.

It is found that those individuals of this fraternity which, though healthy, were characterized by abnormally low blood sugar were individuals with the largest thyroids and largest suprarenals found within the fraternity. And that individuals characterized by abnormally high sugar values usually had the smallest thyroids and suprarenals. Much less definitely the data suggest that the largest gonads, both ovary and testis, are to some extent associated with the low sugar and with large size of thyroid and suprarenal. The sizes of two or three different organs are therefore correlated with each other; and each is correlated with a type of abnormal sugar level, high or low. But the exact amount of this correlation is in no case readily calculated or expressed numerically,

since the data must be subdivided for sex, and each sex must again be subdivided into a healthy group and two additional groups of diseased individuals. The weights of other organs show that other associations than those mentioned above were not found. It therefore seems advisable to attempt the presentation of the data in tabular form only.

Consideration of data. It should be made clear that the birds studied were hybrids, and that their parents were of different genera. The sire was a *Spilopelia suratensis;* the dam was a hybrid from three species of *Streptopelia* (*alba-douraca-risoria*). The offspring were all reared in the same year, were all mature, and aged 6.2 to 16.3 months when killed for examination. The sugar determinations were all made between June 17 and August 17 and all the birds were killed within one to twenty-two days after their blood sugar was determined. Some of the birds suffered a distinct loss in body weight during the few days which in some cases intervened between the drawing of blood samples from the heart and the weighing of the organs at necropsy. This loss of weight—exactly known to us in all cases—probably caused a decrease in weight of the thymus gland since this organ has been found to be quickly diminished under similar conditions. The body weight tabulated is of course that found at necropsy, though we believe that the more nearly uniform body weights found at the time of the sugar determinations form a better general basis of comparison. We further note that weights of these thyroids were obtained at essentially the same season and are therefore wholly comparable. This is a matter of importance, since Riddle (unpublished data) has found that these organs in pigeons probably undergo a seasonal variation in size.

The data are first subdivided on the basis of sex. This division is indicated by the probability that the sexes have thyroids of unequal size. In the present data these organs in the healthy males average 13.9 mgm. and 13.5 in the females; but if a single and quite abnormal thyroid weight (no. 22) be discarded the female average is only 11.5 mgm. In the two diseased groups there is a rather similar difference. Again, comparisons of gonad size make this division of the sexes obligatory. It is further necessary to treat separately the healthy, the tuberculous, and the worm-infested (*Ascaridia*) within each sex; for it has been shown by Riddle (1) that in these two last-named groups the suprarenals often or usually undergo enlargement. The present data also illustrate this fact.

In the further subdivision of the data for presentation in table 1 we have utilized the fact that the average sugar value found for the twenty healthy birds of this fraternity is 163 or 164 mgm. per 100 cc. of blood (9 males = 163; 11 females = 164). Values approaching this figure may therefore be considered "medium" or normal values. In general, too.

the attempt is made to separate the three extreme blood sugar values, both low and high, from the remainder of the group. If a typical organ size is associated with low and another organ size with high blood sugar such an association can of course be most readily observed by separating the extreme values from the mass of normal values.

When the several members of this fraternity are grouped within the various subdivisions in the order of their blood sugar values, and these latter are accompanied by the weights of the various organs of the bird (table 1), the following associations become evident: The birds with abnormally low blood sugars had *large* thyroids and large suprarenals; those with abnormally high sugars had *small* thyroids and small suprarenals. This order is followed closely in the various groups of "healthy" birds—both in males and in females. For birds bearing *Ascaridia* the same rule holds for the males but not for the females, though in this case there is no "high" sugar group with which the "low" may be compared.

It is practically certain that the indicated association of thyroid and suprarenal size with blood sugar is not a matter of chance. Among the healthy birds, both male and female, it will be observed that in the case of the thyroid there is no overlap of figures within the "low" and "high" groups; i.e., the highest thyroid weight of the "high" sugar group is lower than the lowest thyroid weight of the "low" sugar group. This absence of overlap also holds true for the thyroids of the "low" sugar group as against the "medium" sugar group except for a single evidently abnormal thyroid (23.3 mgm.) in the female group; there is no exception in the group of males. For the suprarenals the individual exceptions are less rare, but the group averages for suprarenal are nearly consistent. The only real exception is in the case of the worm-infested females, where the comparison is between the "low" and "medium" sugar groups only; elsewhere the higher average suprarenal weight is associated with lower blood sugar. It is notable that among the 10 worm-infested birds there is practically no instance of abnormally "high" blood sugar; and among the 7 tuberculous birds there is no instance of a "medium" or normal sugar value.

Among the healthy birds, both male and female, the data suggest that birds of "low" sugar groups have somewhat larger gonads than do the birds of "high" and "medium" sugar groups. This relationship largely holds in comparisons of groups, but some notable exceptions are found for individuals within the groups. Gonad size, however, is so susceptible of change under a variety of conditions—more particularly in females—that we do not feel justified in stressing the value of these data. In connection with this apparent tendency of well-developed gonads to be associated with thyroids and suprarenals of larger size we further note that the gonads of the worm-infested birds tend to be larger than in the healthy birds; and that elsewhere, with more adequate material, Riddle (1) has shown that the presence of *Ascaridia* in otherwise normal doves and pigeons leads to a marked increase in

TABLE 1

On a relation of thyroid and suprarenal size to blood sugar concentration in a fraternity of generic hybrids

GROUP	SUGAR VALUE	NUMBER	BLOOD SUGAR	WEIGHT OF:				AGE (IN MONTHS) WHEN KILLED	WEIGHT OF:				LENGTH OF INTESTINE
				Thyroid		Suprarenals	Body		Gonads	Thymus	Liver	Spleen	
				Milligram	Tenthousandths of body weight								
						mgm.	*gm.*		*mm.*	*mm.*	*gm.*	*mm.*	*cm.*
Males (healthy)	Low	1	130	15.4	10.20	12.6	151	9.2	500	2067	3.51	335	55
		2	145	19.9	14.63	9.4	136	13.3	821	757	2.21	182	43
		3	150	19.3	13.69	12.0	141	14.6	718	753	2.44	295	51
		Ave.	*142*	*18.2*	*12.73*	*11.3*	*143*	*12.6*	*680*	*1192*	*2.72*	*271*	*50*
	Med.	4	163	14.1	10.29	8.1	137	11.7	761	404	3.50	215	42
		5	165	11.1	6.69	9.0	166	12.6	485	923	2.58	478	55
		6	168	8.1	5.36	9.1	151	12.5	718	569	2.93	162	52
		Ave.	*165*	*11.1*	*7.35*	*8.7*	*151*	*12.3*	*655*	*632*	*3.00*	*285*	*50*
	High	7	178	11.5	7.67	9.1	150	11.0	478	305	2.78	350	46
		8	180	13.5	9.64	7.2	140	11.0	290	950	2.34	370	48
		9	185	12.5	8.87	8.7	141	12.7	604	965	2.14	145	45
		Ave.	*181*	*12.5*	*8.68*	*8.3*	*144*	*11.6*	*457*	*749*	*2.42*	*288*	*46*
Males (Ascaridia)	Low	10	125	11.8	7.97	9.1	148	11.7	896	1823	2.19	253	48
		11	128	13.2	9.04	11.6	146	12.9	717	606	2.55	241	51
		12	138	11.0	7.75	15.0	142	13.7	741	564	2.91	195	51
		Ave.	*130*	*12.0*	*8.28*	*11.9*	*145*	*12.8*	*785*	*998*	*2.55*	*230*	*50*
	Med.	13	148	12.8	8.65	10.1	148	14.3	818	756	2.33	206	46
		14	175	9.7	7.19	8.4	135	15.2	410	354	1.72	205	41
		Ave.	*162*	*11.3*	*7.96*	*9.3*	*142*	*14.8*	*614*	*555*	*2.02*	*206*	*43*
								8.9	65	1038	2.39	365	51

Females (healthy)	Low	15	125	14.5	10.82	9.9	134	8.9	65	1038	2.39	365	51
		16	135	11.7	7.45	10.4	157	16.2	68	825	2.65	273	52
		17	145	17.6	10.86	11.6	162	13.0	163	1050	2.83	390	51
		Ave.	*135*	*14.6*	*9.66*	*10.6*	*151*	*12.7*	*99*	*991*	*2.62*	*343*	*51*
	Med.	18	155	10.5	7.78	7.6	135	9.1	71	336	1.81	235	46
		19	160	11.4	8.32	9.0	137	6.4	43	2151	2.76	514	52
		20	168	10.6	8.62	8.6	123	13.6	103	470	1.88	264	43
		21	175	8.4	6.32	9.9	133	6.8	38	274	1.91	273	46
		22	180	23.2	17.84	11.2	130	7.7	37	665	2.59	330	45
		Ave.	*168*	*12.8*	*9.70*	*9.3*	*132*	*8.7*	*58*	*779*	*2.19*	*323*	*46*
	High	23	185	11.0	7.75	10.2	142	12.5	47	438	3.32	430	46
		24	205	11.2	7.42	9.9	151	11.5	94	667	1.80	253	47
		25	208	8.5	5.94	8.0	143	8.1	37	1554	2.65	535	60
		Ave.	*199*	*10.2*	*7.03*	*9.4*	*145*	*10.7*	*59*	*890*	*2.59*	*406*	*51*
Females (Ascaridia)	Low	26	135	10.7	6.69	9.0	160	16.3	134	1511	2.65	490	61
		27	135	10.9	7.84	8.7	139	14.6	103	410	2.40	399	56
		28	144	10.5	7.66	8.7	137	16.6	129	850	1.89	410	53
		Ave.	*138*	*10.7*	*7.38*	*8.8*	*145*	*15.8*	*122*	*924*	*2.31*	*433*	*57*
	Med.	29	158	10.7	6.69	10.5	160	14.9	225	1504	2.22	723	52
		30	172	12.5	8.39	8.6	149	13.8	129	415	2.83	340	56
		Ave.	*165*	*11.6*	*7.48*	*9.6*	*155*	*14.4*	*177*	*960*	*2.53*	*532*	*54*
Tuberculous (♂ and ♀)	Low	31	130	11.9	7.93	11.6	150	9.2	536	2204	3.01	305	43
		32	143	11.2	10.98	32.9	102	11.2	211	Trace	4.92	223	48
		33	145	18.8	15.54	22.2	121	12.3	230	(200)	5.54	532	50
		34 ♀	150	13.5	12.39	31.4	109	6.4	♀ 58	Trace	5.42	1270	55
		35	150	11.6	10.74	21.5	108	11.3	135	Trace	5.14	303	45
		Ave.	*144*	*13.4*	*11.36*	*23.9*	*118*	*10.1*			*4.81*	*527*	*48*
	High	36 ♀	183	10.6	9.72	32.7	107	12.8	♀ 39	(200)	3.57	328	43
		37	200	11.1	9.40	11.8	118	6.2	317	598	3.17	490	43
		Ave.	*192*	*10.9*	*9.65*	*22.3*	*113*	*9.5*			*3.37*	*409*	*43*

suprarenal size; it is possible that the thyroid then shows a similar enlargement (unpublished data). These considerations therefore reinforce the insufficient evidence given in the table and suggest that larger gonads accompany that type of enlarged thyroids and suprarenals which is associated with low blood sugar. It is probable, however, that the slightly older average age of the worm-infested birds partially accounts for larger gonad size in this subdivision of the present data. In the group of tubercular males it can be seen that the testes are notably diminished in size; this also accords with extensive experience.

In one column of the table the weights of the thyroid have been divided by the body weight and the quotient expressed in terms of ten-thousandths of the body weight at necropsy. Examination of the figures thus obtained makes it clear that the above described relation of thyroid size to the type of blood sugar—low or high—remains practically the same whether body weight is or is not considered. We have also made the same calculations using the body weights obtained at the time the sugar determinations were made and find there also no change due to body weight. It seems necessary to conclude that the relation of the size of thyroids and suprarenals to the type of blood sugar can be attributed neither to chance nor to the body weight of the animal.

In the above calculation it is shown that among the members of a single fraternity of pigeons the ratio of size of thyroid to size of body is relatively high for individuals having abnormally low sugar values (11.20:10,000) and low for those with abnormally high sugar values (7.86:10,000). One may therefore ask whether pigeon species with low normal values show higher or lower thyroid-body ratios than other pigeon species with high *normal* sugar levels. We have at hand some data suitable for a solution of this problem; and in the three or four species covered by these data we find that such a relation does not exist. *Turtur orientalis* and *Columba domestica* have high normal sugars (188 and 181 mgm.) with thyroid-body ratios of 9.42 and 14.59 respectively. *Streptopelia risoria* has a low normal sugar (149 mgm.) with a ratio of 10.49. A group of hybrids (*T. orientalis* × *St. alba*) with an intermediate normal sugar value (167 mgm).—only slightly higher than the hybrids described here—gives a thyroid-body ratio of 12.42. The ratio for all similar healthy hybrids of the present study is 9.30:10,000. It is true, however, that the ages of the birds included in the above calculations differ widely in the different species; and that the range of the size variation of the thyroids in those groups (6 to 49 individuals in each) derived from different strains, or at least from different parents, is much greater than in the present more homogeneous series. Other disturbing factors enter into the calculations. It seems probable, however, that the above species showing high or low normal blood sugar do not vary in any constant manner in the relative size of their thyroids.

It has not been possible to make another test of the relation described in this paper of thyroid and suprarenal size to *abnormally* low and high sugar values. We have obtained blood sugar values from no other sufficiently large group of brothers and sisters. A similar comparison between individuals of the same species or race, not full brothers and sisters or at least closely related, can not be made because of a probable variation of the thyroid in different strains and families of the same race or species.

Discussion. The absence of histological data for the thyroids and suprarenals of the individuals of this fraternity necessarily restricts the value of a consideration of the meaning of the large size of these

organs in the low sugar group, and of their small size in the high sugar group. The fact of this size relationship is the point established by this paper. The suprarenal size relationship observed in this particular and apparently unique situation is the reverse of that to be expected from earlier observations in other forms and also in the pigeon. Riddle (1) has shown that these glands enlarge at each ovulation period, and Honeywell and Riddle (2), (3) have shown that the blood sugar is then increased coincidentally with that increase in size. There are of course no ovulation periods involved in the present data. Thyroid hyperactivity, too, is generally considered as tending toward the production of hyperglycemia.

We leave all discussion of the point raised above with the following observation: If we suppose the larger suprarenals of this series to represent enlargements of cortex, and the enlarged thyroids to represent deficient (goitrous?) glands, then their observed condition is in harmony with the suggestion of Marine and Baumann (4) that the cortex opposes thyroid activity; and if this opposition was quite successful in the cases now being considered the condition of both glands becomes consonant with low sugar, since a reduced thyroid activity prevails and there is no increase of medullary tissue to raise the blood sugar. In the opposite case—of small glands associated with high blood sugar—one may suppose that these suprarenals possess but slight amounts of cortex and so offer slight opposition to the thyroids; and that these thyroids, though small, were highly active (being little opposed by cortex); the high blood sugars associated with them would then appear as thyroid hyperglycemias. This suggestion has perhaps only the merit of a conceivable explanation of a difficult point.

The wide range of blood sugars found among the healthy brothers and sisters of this fraternity is a notable fact. One may first wish to know whether this diversity is an outcome of the *hybrid* nature of these individuals. The behavior of blood sugar values in heredity, including its behavior in the present family of hybrids, has been elsewhere described by Riddle and Honeywell (5). We may therefore here note only that the three species represented in the dam have normal sugar values apparently close to 149 mgm. per 100 cc.; that of the sire a value of 170 or 175. The influence of heredity would be toward the production of larger numbers of offspring with sugar values between the normals for the species crossed; but since the mother was hybrid, and multiple factors are conceivably concerned in the inheritance of organ size, the production of variants beyond these normal species limits is also possible. If such an hereditary basis were fully established as accounting for the diversity observed here this fact would not establish a difference between the present data and the rather similar variation found among individuals

of human or other mammalian species. These latter may also have an hereditary basis.

The present results offer a foundation of fact which should contribute to an understanding of the general observation that some normal individuals of any race, strain or species examined consistently have blood sugars higher or lower than that considered normal for the race. Evidence that individual pigeons thus continuously and rather consistently maintain different sugar levels has been presented by Honeywell (6). It is now made probable that the sugar levels in such observations are associated with size types of certain incretory organs. The thyroid and suprarenal are cases in which this size relationship has now been observed.

SUMMARY

In a group of brother and sister hybrids it has been found that the group of birds giving abnormally low blood sugars had large thyroids and large suprarenals. Those giving abnormally high blood sugars had small thyroids and small suprarenals. Less conclusive evidence indicates that large gonads are associated with the large thyroids.

An adequate interpretation of this relationship is not attempted. A suggestion is made concerning this sugar-glandular association.

The results probably assist an understanding of cases of normal individuals of the same race or strain which show consistently low or consistently high blood sugar.

The data supply an unusual indication that thyroid and suprarenal are both in some way concerned in the establishment of the normal concentration of sugar in the blood.

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COMPARATIVE STUDIES OF DIGESTION

III. Further Observations on Digestion in Coelenterates

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The digestive processes in coelenterates are characterized by their slowness and in general appear to parallel the activity and metabolic requirements of the various members of this phylum. Thus, enzyme extracts, prepared from the hydroids tubularia and Obelia, are less effective in digesting proteins than are those obtained from Metridium or the still more active coelenterates, Stomolophus and Physalia.

In a previous communication, Bodansky and Rose (1) presented data showing that extracts prepared either from the siphons of the Portuguese-man-of-war (Physalia arethusa) or from the mesenteric filaments of the jellyfish (Stomolophus meleagris) are capable of digesting gelatin both on the acid and alkaline side of the iso-electric point. Two optima of digestion were obtained, one at a pH of 3.0 to 3.5 and the other in an alkalinity equivalent to pH 7.3 to 7.6. These observations indicate the presence of a peptic as well as of a tryptic enzyme. It is to be emphasized, however, that trypsin is the predominant proteolytic enzyme in these coelenterates. In addition to pepsin and trypsin, rennin, amylase, maltase and lipase were found to be present both in Physalia and in Stomolophus.

The present investigation was undertaken with the object of extending our knowledge of the digestive enzymes of coelenterates and to determine whether extracellular enzymatic digestion occurs in certain of these animals. In 1874, Claus (2) observed the presence of foreign particles in the interior of the endoderm cells of siphonophores and suggested that food particles might be taken up directly by these cells. In his study of the histology of Hydra fusca, Parker (3) reported that in this species digestion was accomplished within the cell. The subject was studied by Metchnikoff (4) who observed that the endoderm cells of a number of coelenterates enmeshed particles of carmine by means of pseudopoda. He therefore inferred that food particles were similarly absorbed and subsequently digested within the cells; in other words, that the process of digestion in coelenterates is essentially intracellular and the same as in amoeba.

At about the same time, Krukenberg (5) demonstrated that food, placed in the gastric cavity of actinia, was digested only when the mesenteric filaments came in direct contact with the food particles. Krukenberg therefore concluded that digestion occurs at the surface of contact between the filament and the food. While admitting the absence of extracellular digestive secretions, he objected to Metchnikoff's hypothesis, stating that no analogy could be drawn from the latter's experiments with carmine and the actual process of digestion.

Later, Mesnil's observations (6) showed that digestion in actinia is not so much dependent upon the contact of the filaments with the food as upon the absorption of the foodstuffs within the amoeboid cells lining the mesenteric filaments. In a paper which appeared in 1907, Jordan (7) reasserts that slight digestion occurs in the gastric cavity of actinia. Reference is made to Greenwood's observation (8) that partial proteolytic digestion takes place in the gastric cavity of hydra. The bulk of the evidence, however, appears to indicate that digestion in coelenterates is intracellular. Nevertheless, it should not be overlooked that relatively few species of this phylum have been studied. As pointed out by Hickson (9), there exists the possibility that within the group of coelenterates some members may show the development of extracellular digestion.

In a number of our earlier experiments with Stomolophus, some evidence was obtained indicating the presence of digestive enzymes (trypsin and amylase) in the gastric cavity of animals containing food or food remnants. Although the gastric fluid was filtered, it was difficult to determine whether these enzymes were extracellular in the sense that they had been secreted by the digestive tissues under the stimulus of the food. There is the possibility that the enzymes may have had their origin in the food previously contained in the digestive cavity.

We have been able to investigate this problem under more favorable experimental conditions at the Marine Biological Laboratory in Woods Hole, Mass., using Metridium marginatum.

Intracellular digestion. In the study of intracellular enzymes, extracts were prepared by grinding with sand the mesenteric filaments and scrapings from the walls of the gastro-vascular cavity of Metridium in the presence of toluene and water or glycerol. For the determination of proteolytic digestion, we employed the method of Dernby (10) previously used in our studies on coelenterates (1) and on elasmobranchs and teleosts (11).

The results of the experiments with Metridium show that proteolytic digestion in this coelenterate is very slow, that pepsin is present in but minute traces and that trypsin is the predominant proteolytic enzyme. As in the case of other coelenterates, the digestive tissues of Metridium possess weak rennitic action.

Diastatic activity in Metridium was demonstrated by allowing tissue preparations to act on dilute starch solutions in the presence of toluene as a preservative. The reducing power of the digests was determined at the beginning and at definite intervals during the experiment by means of the well-known Folin-Wu blood sugar method. By using the disaccharides as substrates, we have determined the presence of maltase and the absence of invertase and lactase in Metridium.

Enzymatic hydrolysis of fats by the tissue suspensions of Metridium was determined in a number of experiments. The hydrolysis of the ester, ethyl butyrate, was likewise observed.

Extracellular digestion. Repeated experiments have shown that the fluid removed from the gastro-vascular cavity of Metridium, after filtration, exerts no measurable digestive effect on proteins. On the other hand, such fluid, if unfiltered, is capable of digesting gelatin in neutral or slightly alkaline solutions. Unfiltered gastro-vascular juice likewise exerts measurable diastatic effect. On the basis of these results, we are inclined to the view that the enzymes ordinarily found in the unfiltered fluid are associated, for the most part, either with food remnants or with fragments of the mesenteric filaments.

At the suggestion of Prof. G. H. Parker, the gastric fluid was collected from some animals after suspending them from boards and allowing them to drain for several hours. In one experiment, the gastro-vascular fluid was removed from twenty-four metridia by means of a pipette. The animals were then replaced in running sea water where they remained for 48 hours. At the end of this period a small piece of boiled crab meat was placed in the digestive cavity of each metridium. After remaining in runinng sea water for 6 hours longer, the metridia were removed and allowed to drain on sloping boards for several hours. Subsequently, the animals were cut horizontally and the gastro-vascular fluid allowed to collect in beakers. This juice, both filtered and unfiltered, was found to contain small amounts of proteolytic and amylolytic enzymes. Whereas the unfiltered fluid was quite effective in digesting gelatin, the proteolytic action of the filtered fluid was much less marked.

The experimental evidence upon which extracellular digestion in coelenterates is denied, is partly that adduced microscopically by Metchnikoff and partly the demonstration by Mesnil and other investigators that the digestive enzymes are present in the cells lining the mesenteric filaments of certain coelenterates. It is to be appreciated that in these animals, digestion as a whole is exceedingly slow. Therefore, the amount of enzyme to be found in the digestive cavity, at any one time, would be very small and impossible of detection by the methods available to the earlier investigators. The localization of enzymes within the cells of the digestive tissues in coelenterates does not necessarily prove that in

these animals digestion is exclusively intracellular. It is to be recalled that the source of active commercial enzyme preparations, such as pepsin, is the gastric mucosa rather than the gastric juice.

It has been generally overlooked that within the limits of our present knowledge, there is but little apparent difference in the mechanism of enzymatic digestion in the animal series. Even in protozoa, the enzymes, liberated from the cytoplasm, must obviously traverse a layer of water which separates the food in the digestive vacuole from the cell protoplasm. In a sense, digestion here is really extracellular. The engulfment of food particles within the cells in these forms as well as in sponges and certain of the coelenterates serves only to prevent the excessive dilution and loss of enzyme that would occur otherwise. In those coelenterates where the oral aperture becomes contracted following the admission of food into the digestive cavity as in Metridium and possibly in Physalia, the loss of enzyme by dilution and washing out by the circulating sea water is largely prevented.

SUMMARY

In Metridium marginatum the following enzymes have been found: trypsin, rennin, amylase, maltase and lipase. Pepsin is present in very small amounts. Our results indicate the absence of lactase and invertase.

Unfiltered gastro-vascular fluid contains both trypsin and amylase. In a number of experiments, we have detected small amounts of these enzymes occurring extracellularly.

It is concluded that in Metridium the digestive enzymes occur, for the most part, intracellularly. However, partial disintegration of foods may take place within the digestive cavity as a result of autolytic changes and to some extent by extracellular enzymes having their origin in the cells lining the mesenteric filaments.

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THE OUTPUT OF EPINEPHRIN FROM THE ADRENAL GLANDS DURING CEREBRAL ANEMIA

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It has been conclusively demonstrated that the spontaneous liberation of epinephrin from the adrenal glands is dependent upon the integrity of the nerve supply to the glands (1), (2). A central mechanism capable of sustaining this secretion has been shown to be located in the upper thoracic cord (3), (4). It is of course probable that another center (or centers) may exist higher up, whose function is more or less associated with that of the mechanism in the upper thoracic cord, but there is at present no conclusive evidence of this.

The output of epinephrin from the adrenals in animals after destruction of the cerebral cortex (with a curette) or in animals rendered insensitive by increased intracranial pressure, has usually been found to be of the same order of magnitude as when the rate of epinephrin secretion is measured under ether or urethane anesthesia, without mechanical disturbance of the brain (5), (6).

Elliott (7) observed that exhaustion of the epinephrin store in the adrenals by electrical excitation of afferent nerves occurs if the brain-stem is transected just above the anterior corpora quadrigemina, but does not occur if the cord is transected anywhere below the level of the vasomotor center in the medulla. The experiments are complicated by the fact that under the influence of the anesthesia, etc., exhaustion of the store proceeds steadily in the absence of electrical excitation of nerves, and it may be difficult to make exact allowance for this. Elliott believed the exhaustion developed more rapidly than would have been the case with ether anesthesia alone. He made no observations on the rate of liberation of epinephrin from the adrenals, and since it has never been proved that the exhaustion of the store of epinephrin is due to increased liberation, these observations have no direct bearing on the question.

Cannon and Rapport (8) conclude that the reflex center for adrenal secretion is located near the upper or front edge of the floor of the fourth ventricle. Unfortunately, they rely entirely upon changes in the rate

of the denervated heart, interpreting all such changes as indications of increased or diminished rate of epinephrin output.

This method was employed recently by Searles (9) in an attempt to prove an increase in the rate of epinephrin secretion from the adrenals, during asphyxia or sciatic stimulation, in dogs. The writer states that it seems to him "a safe inference that more adrenin is secreted as the result of asphyxia or sciatic stimulation" and he "concurs with the statement made by Cannon, that the necessary technique for demonstrating reflex adrenin discharge by the denervated heart method is so simple that anyone inclined to doubt that more adrenin is secreted in consequence of reflex or other stimulation, may readily make the test."

It is surprising how easily some writers are convinced. Stewart and Rogoff (10) did make the test, although they did not think that exact information on the rate of epinephrin secretion from the adrenals could be obtained in this way. They found, as was later confirmed by Cannon, that acceleration of the denervated heart can be caused by asphyxia or sensory stimulation in the absence of the adrenal glands as well as when they are secreting epinephrin. Since Cannon has observed that this reaction is also an indication of activity of the liver and thyroid, and is obtainable on injection into the circulation of many substances, especially extracts of salivary glands, it is evident that this reaction is very far from being specific for epinephrin and that results obtained with this method can not yield reliable information on the rate of secretion of epinephrin from the adrenals. Starving an animal can hardly be expected entirely to eliminate all sources of error inherent in the method. If Searles, as he should have done, had attempted to determine the amount of epinephrin given off, without and during asphyxia or sensory stimulation, he would have found that the denervated heart reaction, as employed by him, could not be relied upon for such quantitative information.

It has been alleged that certain vasomotor phenomena associated with anemia of the brain and bulb induced by occlusion of the head arteries are dependent upon increased secretion of epinephrin from the adrenals, although estimations of the rate of secretion were not made (11). All of these phenomena have been observed by Rogoff and Coombs (12) in animals in the absence of epinephrin secretion as well as when the adrenals were not interfered with. We pointed out, however, that although the vasomotor reactions do not depend upon epinephrin secretion, it is nevertheless conceivable that the profound disturbance brought about in the upper part of the central nervous system by occluding the head arteries might affect central mechanisms concerned with adrenal activity, together with the other centers in the anemic area, and thus alter the rate of epinephrin liberation from the glands.

Three experiments on cats were reported in an earlier paper from this laboratory (4) in which the rate of epinephrin output from the adrenals was examined 15 to 30 minutes after permanent cerebral anemia was induced by ligating the head arteries. No significant alteraton in the rate of epinephrin secretion was demonstrated in these experiments. During the course of the work by Miss Coombs and myself we made two preliminary experiments to investigate the possibility that a transient

change in the rate of epinephrin secretion might occur during the period when the vasomotor center is responding to the powerful stimulation initiated by the anemia. In one of these experiments a moderate increase in the epinephrin output was found, although we pointed out that this was not responsible for the vasomotor phenomena elicited as a result of cerebral anemia.

In this paper are reported the results of further investigation of this question. All the experiments were performed on cats. The head arteries were prepared for clipping according to the method employed by Stewart, Guthrie and Pike (13), with the precautions described in the recent paper by Miss Coombs and myself (12). The adrenal vein blood was collected through a cannula in a "cava pocket," and assayed on rabbits' intestine segments. Determinations of the rate of epinephrin output were made by assaying specimens obtained before inducing cerebral anemia and again on adrenal blood specimens collected at varying intervals after the beginning of occlusion of the head arteries.

A majority of the experiments showed a definite increase in the rate of epinephrin output from the adrenals at the time when the anemia was producing the usual vasomotor and other responses. That the increased rate of epinephrin secretion is not responsible for the vasomotor phenomena is evident from these experiments since at the time that the blood pressure changes were taking place the adrenal epinephrin was not passing into the circulation of the animal but was being collected through the cannula in the cava pocket. Additional proof was found, in this series of experiments, of the fact that the so-called "dissociation" or "double" curve often seen during occlusion of the head arteries can be obtained at the time when no epinephrin from the adrenals can be entering the circulation, and that the second rise in the blood pressure is therefore not caused by augmented secretion, as has been supposed.

Thus in cat 804, occlusion of the head arteries was followed by a double rise in the blood pressure, in two observations, at times when the cava pocket was shut off (just beneath the liver) for the collection of adrenal blood, (fig. 1, obs. *5* to *9* and *12* to *14*).

Condensed protocol. Cat 804; male; weight, 2.84 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae, prepared head arteries for occlusion, then prepared cava pocket.

11:25 a.m.; blood pressure 130 mm. Hg

11:30 a.m.; first adrenal blood specimen, 1.05 grams in 30 seconds (2.1 grams per minute)

11:30½ a.m.: second specimen, 3.2 grams in 2 minutes (1.6 grams per minute)

11:34 a.m.; blood pressure 72 mm. Hg; began occlusion of head arteries

11:34½ a.m.; third specimen, 1.05 grams in one minute

11:35½ a.m.; fourth specimen, 1.55 grams in 90 seconds (1.03 grams per minute)

11:37 a.m.; released head arteries; blood pressure, 60 mm. Hg

11:37½ a.m.; fifth specimen, 1.6 grams in 3 minutes (0.53 gram per minute)

11:40½ a.m.; sixth specimen, 1.9 grams in 3 minutes (0.63 gram per minute)

11:48 a.m.; blood pressure, 42 mm. Hg; occluded head arteries for 2 minutes; blood pressure after end of occlusion, 26 mm. Hg

Indifferent blood obtained.

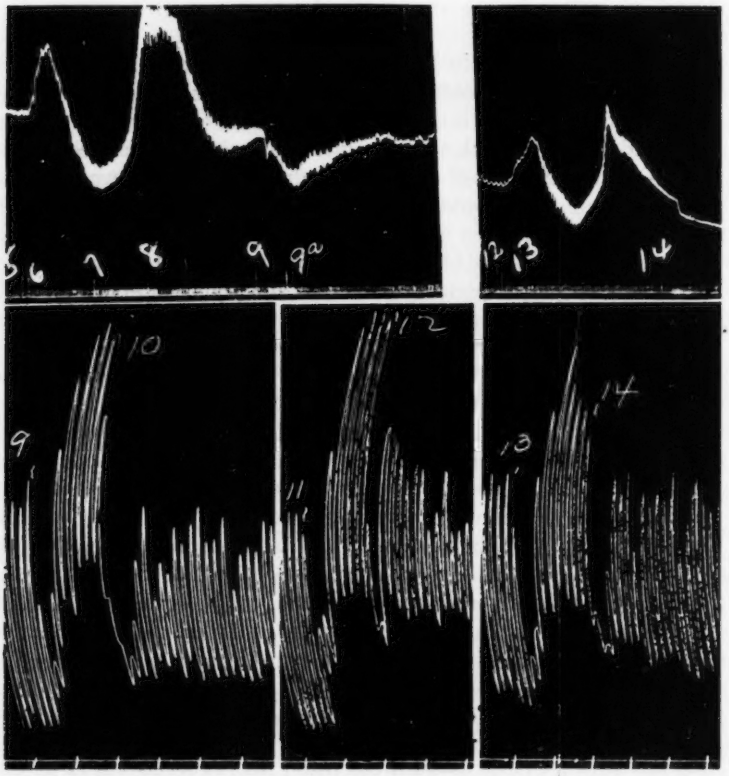

Fig. 1. Upper; blood pressure tracing; cat 804. At *5* and *6*, head arteries occluded; *7*, began collection of third adrenal blood specimen; *8*, began collection of fourth specimen; *9*, head arteries released; *9a*, began collection of fifth specimen; *12* to *14*, occluded head arteries for two minutes.

Lower; intestine tracings; bloods from cat 804. At *9*, *11* and *13* Ringer was replaced by indifferent blood and this at *10* by indifferent blood to which was added adrenalin to make a concentration of 1:5,000,000, at *12* by the second adrenal blood specimen and at *14* by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000. All the bloods were diluted with 3 volumes of Ringer, the adrenalin bloods after adding the adrenalin. As in all other intestine tracings the time is in half minutes. The base line of time trace (seconds) in the upper tracing, corresponding to zero pressure, was moved up 15 mm. and the entire figure then reduced to four-fifths.

In the past few years we have already published many intestine tracings illustrating the manner of assay for epinephrin in adrenal vein blood. Therefore only a few samples of the intestine tracings from this and other

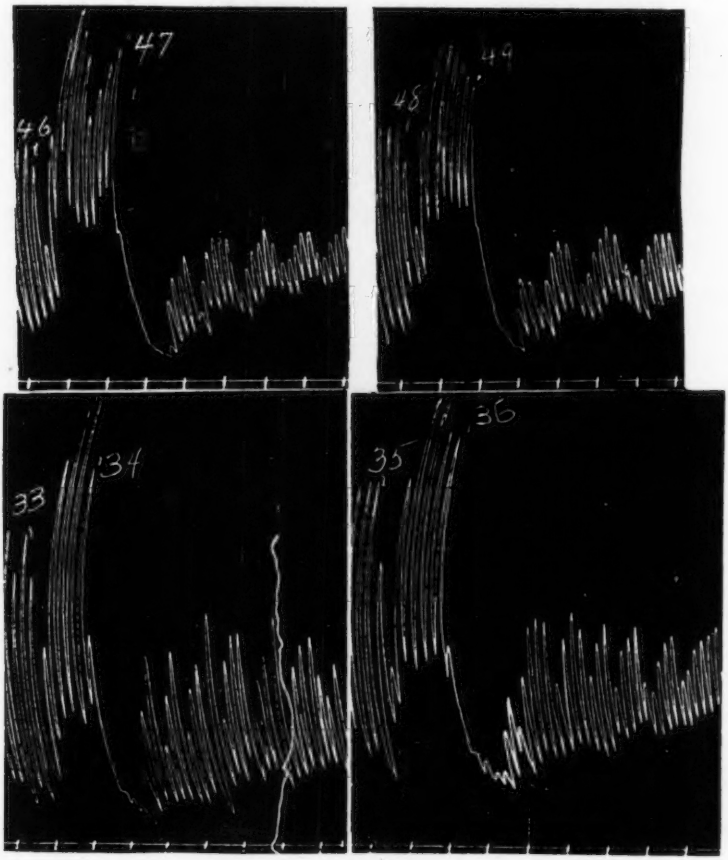

Fig. 2. Intestine tracings; bloods from cat 804. At *33*, *35*, *46* and *48* Ringer was replaced by indifferent blood and this at *34* and *49* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000, at *36* by the fourth adrenal blood specimen diluted with one volume of indifferent blood and at *47* by the sixth specimen diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes of Ringer, the adrenalin bloods after adding the adrenalin and the adrenal specimens after adding the indifferent blood. Reduced to three-fourths.

experiments are reproduced, as it is of course impossible to include the large number of tracings necessary to demonstrate a complete assay of

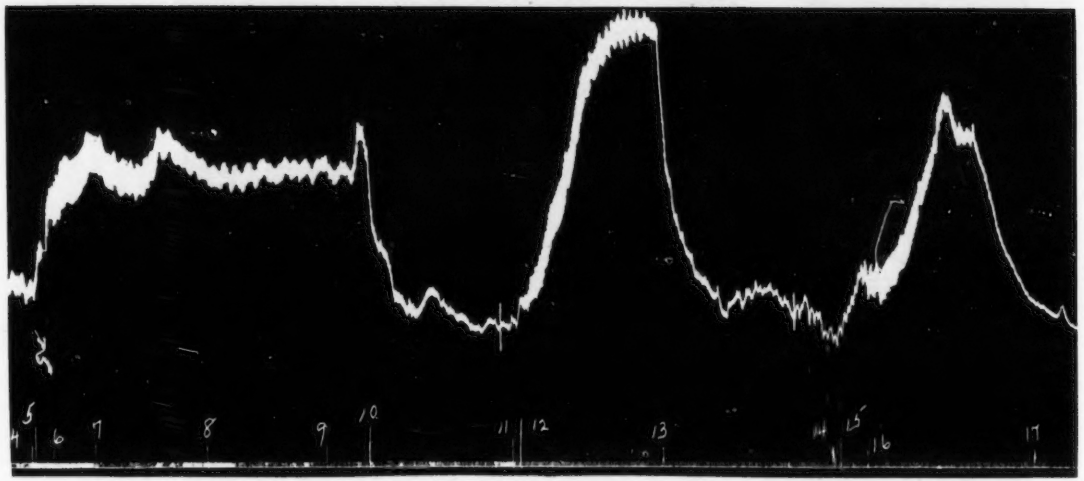

Fig. 3. Blood pressure tracing. Cat 806. At *4* and *5*, head arteries occluded; *6*, began collection of third adrenal blood specimen; *7*, began collection of fourth specimen; *8* to *9*, collection of fifth specimen; *10*, released head arteries; *11* to *13*, occluded head arteries for 2 minutes; *14* and *15*, head arteries occluded; *16*, began collection of sixth specimen; *17*, began collection of seventh specimen. Base line of time trace corresponds with zero pressure. Time in seconds. Reduced to two-thirds.

all the blood specimens. In every case the assay of a blood specimen was confirmed by a number of sets of tracings.

The second specimen, collected before occluding the head arteries, was decidedly weaker than 1:2,500,000 adrenalin, somewhat weaker than 1:5,000,000, somewhat stronger than 1:6,250,000 (fig. 1, obs. *10* to *14*) and decidedly stronger than 1:8,000,000. The specimen was taken at 1:5,500,000, corresponding to an output of 0.0003 mgm. per minute for the cat or 0.00011 mgm. per kgm. per minute.

The third and fourth specimens, collected during the period of occlusion of the head arteries, gave practically the same reaction. They were somewhat stronger than 1:625,000 (fig. 2, obs. *34* and *36*) and decidedly weaker than 1:312,000. They were assayed at 1:600,000, corresponding to an output of 0.0017 mgm. per minute for the cat or 0.0006 mgm. per kgm. per minute.

The blood flow during the collection of the fifth and sixth specimens, just after releasing the head arteries, was very slow and the epinephrin concentration in these specimens very high. Diluted with three volumes of indifferent blood they yielded reactions stronger than 1:1,875,000 adrenalin, decidedly weaker than 1:940,000, and not far from 1:1,250,000 (fig. 2, obs. *47* and *49*). Taking them at 1:1,400,000, i.e., at 1:350,000 in the undiluted blood, we get an output of 0.0015 mgm. per minute or 0.00053 mgm. per kgm. during the collection of the fifth specimen and 0.0018 mgm. per minute for the cat or 0.00063 mgm. per kgm. per minute during the collection of the sixth specimen. In this animal the epinephrin output from the adrenals was increased five to six times during the occlusion of the head arteries.

In cat 806 the blood pressure curves during occlusion of the head arteries were of the ordinary type (fig. 3). After collecting adrenal blood, without occluding the head arteries, two sets of adrenal specimens were obtained, 20 minutes apart, with occlusion of the head arteries during the collection of each set, and a period of occlusion (without collection of adrenal blood) during the interval. An increase of four to five times the initial output was observed during the first period of occlusion, and a smaller increase in the specimens obtained during the later occlusion. We cannot be certain, however, that the smaller increase in output, during the collection of the last set of adrenal specimens, was not a continuation of the effect of the first occlusion, which may possibly have still been wearing off.

Condensed protocol. Cat 806; male; weight 2.94 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae, prepared head arteries for occlusion, then prepared cava pocket.

9:50 a.m.: blood pressure, 100 mm. Hg

9:54 a.m.; first adrenal blood specimen (collected for 30 seconds)

9:54½ a.m.; second specimen, 3.4 grams in 2 minutes (1.7 grams per minute)

9:59½ a.m.; blood pressure, 84 mm. Hg; occluded head arteries
10:00 a.m.; third specimen, 2.75 grams in one minute
10:01 a.m.; fourth specimen, 6.10 grams in 2 minutes (3.05 grams per minute)
10:03 a.m.; fifth specimen, 5.5 grams in 2 minutes (2.75 grams per minute)
10:05 a.m.; released head arteries; blood pressure returned to 68 mm.
10:08½ a.m.; occluded head arteries for 2 minutes
10:22 a.m.; blood pressure, 58 mm. Hg; occluded head arteries
10:22¼ a.m.; sixth specimen, 4.2 grams in 2 minutes (2.1 grams per minute)
10:24¼ a.m.; seventh specimen, 1.8 grams in 3 minutes (0.6 gram per minute)
10:28 a.m.; released head arteries; blood pressure 38 mm. Hg
Indifferent blood obtained.

The second adrenal specimen, collected before occluding the head arteries, was decidedly weaker than 1:3,750,000 adrenalin, somewhat weaker than 1:5,000,000 and somewhat stronger than 1:6,250,000. It was assayed at 1:5,700,000, corresponding to an output of 0.0003 mgm. per minute for the cat or 0.0001 mgm. per kgm. per minute. The blood flow during the collection of the third, fourth and fifth specimens, obtained during occlusion of the head arteries, was practically the same and the epinephrin concentration in these specimens was about the same. They were decidedly weaker than 1:1,250,000 adrenalin, weaker than 1:1,875,000 and stronger than 1:3,750,000. They were taken at 1:2,500,000, corresponding to an output of 0.0011 mgm. per minute for the cat or 0.0004 mgm. per kgm. per minute. The sixth and seventh specimens were collected during the third period of occlusion of the head arteries. The sixth specimen was somewhat weaker than 1:1,875,000 adrenalin and stronger than 1:2,500,000. It was assayed at 1:2,000,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.00034 mgm. per kgm. per minute. The seventh specimen diluted with three volumes of indifferent blood was weaker than 1:2,500,000, stronger than 1:5,000,000 and not unlike 1:4,000,000. It was assayed at 1:4,000,000, i.e., 1:1,000,000 in the undiluted blood, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute. It was observed in some of the experiments, that when the blood pressure, after one or more periods of cerebral anemia, has fallen rapidly to spinal level the epinephrin output becomes considerably diminished. It is conceivable that in such a case the sudden fall in blood pressure may result not only in a failure of the nervous mechanism to sustain the augmented output previously induced by the cerebral anemia but, as is indicated from some of the experiments, a condition of spinal shock similar to that which occurs in acute sections of the cervical cord may develop and result in a considerable diminution of the epinephrin output.

In cat 811 adrenal blood was collected during occlusion of the head arteries and again 12 minutes and 30 minutes after the end of occlusion. The blood pressure before clamping the arteries was 107 mm. of mercury

and rose during the occlusion to a maximum of 204 mm. Before the collection of the last pair of blood specimens the pressure was 52 mm. and fell to 43 mm. at the end of the collection. At this time the arteries were again occluded but there was no vasomoter response.

Condensed protocol. Cat 811, female, weight 2.95 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae

10:00 to 10:22 a.m.; prepared head arteries for occlusion

10:23 to 10:35 a.m.; prepared cava pocket

10:40 a.m.; blood pressure 124 mm. Hg

10:41½ a.m.; first adrenal blood specimen, 2.0 grams in 30 seconds (4.0 grams per minute)

10:42 a.m.; second specimen, 5.0 grams in 2 minutes (2.5 grams per minute)

10:51 a.m.; blood pressure, 107 mm.; occluded head arteries

10:51½ a.m.; third specimen, 4.3 grams in one minute

10:52½ a.m.: fourth specimen, 10.4 grams in 90 seconds (7.0 grams per minute)

10:54½ a.m.; released head arteries; blood pressure returned to 80 mm. Hg

11:06 a.m.; fifth specimen, 0.9 gram in 30 seconds (1.8 grams per minute)

11:06½ a.m.; sixth specimen, 3.35 grams in 2 minutes (1.7 grams per minute); blood pressure 69 mm. Hg

11:18 a.m.; cat struggled slightly, pupils dilated and blood pressure fell to 52 mm.

11:25 a.m.; seventh specimen, 0.45 gram in 30 seconds (0.9 gram per minute)

11:25½ a.m.; eighth specimen, 2.9 grams in 4 minutes (0.73 gram per minute)

11:30 a.m.; blood pressure 43 mm. Hg; occlusion of head arteries yielded no response. Obtained indifferent blood.

The second adrenal specimen, collected before occlusion of the head arteries, was somewhat weaker than 1:10,000,000 adrenalin and not unlike 1:12,500,000. It was taken at 1:12,500,000, corresponding to an output of 0.0002 mgm. per minute for the cat or 0.00007 mgm. per kgm. per minute. The fourth specimen, obtained during the period of elevated blood pressure resulting from occlusion of the head arteries, was decidedly stronger than 1:6,250,0000 adrenalin, stronger than 1:5,000,000 and slightly weaker than 1:3,750,000. It was assayed at 1:4,000,000, corresponding to an output of 0.0017 mgm. per minute for the cat or 0.00057 mgm. per kgm. per minute. The sixth specimen, collected 12 minutes after the end of the occlusion, was decidedly stronger than 1:2,500,000 adrenalin, weaker than 1:940,000 and fully as strong as 1:1,250,000. It was taken at 1:1,250,000, corresponding to an output of 0.0014 mgm. per minute for the cat or 0.00046 mgm. per kgm. per minute. The eighth specimen, collected a half-hour after occlusion of the head arteries, was assayed at 1:3,000,000 adrenalin, corresponding to an output of 0.00024 mgm. per minute for the cat or 0.00008 mgm. per kgm. per minute. During the period of cerebral anemia the rate of output was increased to about eight times the initial rate. It was still high 12 minutes later, but a half-hour after the occlusion the epinephrin output had returned to the same rate as before the arteries were occluded. In two other experiments, to be

mentioned farther on, the epinephrin output at the time that the blood pressure had fallen to spinal level was considerably below the initial rate.

It will be observed that the epinephrin output from the adrenals, in the above experiments, was lower before the head arteries were occluded than the average rate found in cats with the method employed. This may, of course, be merely a coincidence, since these outputs fall within the range usually seen. The possibility is suggested, however, that the manipulations associated with the isolation of the head arteries might in some way be responsible for this, as in the above experiments the arteries were prepared for clipping just before the cava pocket was prepared. The operative procedures were reversed in the following experiments, the cava pocket being made and adrenal blood collected before and after isolating the head arteries, as well as during and after occlusion of the arteries.

In cat 807 spontaneous respiration and corneal reflex were present for nearly 10 minutes after the beginning of occlusion of the head arteries. It is known that total anemia of the bulb is not produced in some cats when the head arteries are occluded (12), (13). The inclusion of all of the arteries, in the clamps, was verified post mortem in this animal. A very good blood pressure rise reaching a maximum of 254 mm. of mercury was present during the first 9 to 10 minutes of occlusion.

Condensed protocol. Cat 807; female; weight 2.45 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae and prepared cava pocket.

8:49 a.m.; first adrenal blood specimen, 2.5 grams in 30 seconds (5.0 grams per minute)

8:49½ a.m.; second specimen, 5.05 grams in 90 seconds (3.4 grams per minute)

8:55 to 9:15 a.m.; prepared head arteries for occlusion

9:20 a.m; blood pressure, 120 mm. Hg

9:21 a.m.: third specimen, 1.95 grams in 30 seconds (3.9 grams per minute)

9:21½ a.m.; fourth specimen, 3.2 grams in one minute

9:28 a.m.; blood pressure, 126 mm. Hg; occluded head arteries

9:28½ a.m.; fifth specimen, 5.55 grams in one minute

9:29½ a.m.; sixth specimen, 7.3 grams in one minute; bulbar function suppressed

9:38½ a.m.: seventh specimen, 0.75 gram in 30 seconds (1.5 grams per minute)

9:39 a.m.; eighth specimen, 1.9 grams in 2 minutes (0.95 gram per minute); blood pressure at end of collection, 51 mm. Hg

Obtained indifferent blood while head arteries and cava pocket were still occluded.

The second adrenal blood specimen, collected before, and the fourth specimen, collected after isolating the head arteries (before occlusion) yielded about the same degree of inhibition of the intestine segment. They were stronger than 1:5,500,000 adrenalin, weaker than 1:3,500,000 and not far from 1:4,300,000. They were assayed at 1:4,000,000, corresponding to an output of 0.0008 mgm. per minute for the cat or 0.00032

mgm. per kgm. per minute. The sixth specimen, collected during occlusion of the head arteries gave a much stronger reaction than the second or fourth specimens, indicating qualitatively an increased epinephrin output since the blood flow was about twice as great during collection of the sixth as the second or fourth specimens. Diluted with two volumes of indifferent blood the sixth specimen was stronger than 1:5,000,000 and not unlike 1:3,500,000. It was assayed at this concentration, i.e., at 1:1,200,000 in the undiluted blood, corresponding to an output of 0.006 mgm. per minute for the cat or 0.0024 mgm. per kgm. per minute, i.e., about seven times the initial rate. The eighth specimen was collected 10 minutes after the sixth, while the head arteries were still occluded but at the time when bulbar anemia was complete and the blood pressure was low. Diluted with three volumes of indifferent blood it was decidedly stronger than 1:3,500,000 adrenalin, weaker than 1:2,150,000 and not unlike 1:2,850,000. It was assayed at 1:2,800,000, i.e., 1:700,000 in the undiluted blood, corresponding to an output of 0.0034 mgm. per minute for the cat or 0.0013 mgm. per kgm. per minute, or about four times the initial rate.

This and another experiment (cat 809) are the only ones in the series in which the augmentation of the epinephrin output from the adrenals, during occlusion of the head arteries, brought the rate very much above the upper limits of the spontaneous secretion usually observed by us. In cat 807 this may be due to the fact that the initial rate was good whereas in the experiments previously mentioned it was low to begin with, possibly as a result of the manipulation during the isolation of the head arteries. However, in cat 809 the initial output was only 0.00011 mgm. per kgm. per minute, whereas after occlusion of the head arteries it rose to 0.001 mgm.

In cat 808 the epinephrin output during occlusion of the head arteries was increased to three times the initial rate, from 0.00025 mgm. to 0.00075 mgm. per kgm. per minute. During isolation of the head arteries an excess of ether was administered and it was necessary to resort to artificial respiration for a while to resuscitate the animal.

Condensed protocol. Cat 808; female; weight 3.17 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae and prepared cava pocket.

9:15½ a.m.; first adrenal blood specimen, 1.4 grams in 30 seconds (2.8 grams per minute)

9:16 a.m.; second specimen, 5.0 grams in 2 minutes (2.5 grams per minute)

9:20 to 9:43 a.m.; prepared head arteries for occlusion

9:45 a.m.; blood pressure, 134 mm. Hg

9:46 a.m.; third specimen, 1.95 grams in 30 seconds (3.9 grams per minute)

9:46½ a.m.; fourth specimen, 6.3 grams in 2 minutes (3.15 grams per minute)

9:50 a.m.; blood pressure, 113 mm. Hg

9:53 a.m.; occluded head arteries
9:53¼ a.m.; fifth specimen, 1.65 grams in 30 seconds (3.3 grams per minute)
9:53¾ a.m.; sixth specimen, 8.7 grams in 2 minutes (4.35 grams per minute)
9:57 a.m.; released head arteries; blood pressure, 70 mm. Hg, fell within a few minutes to 44 mm. Hg. Obtained specimen of indifferent blood.

The second adrenal blood specimen, collected before isolating the head arteries, was stronger than 1:3,750,000 adrenalin (fig. 4, obs. *60* and *62*), decidedly weaker than 1:1,875,000, and weaker than 1:2,500,000. It was assayed at 1:3,250,000, corresponding to an output of 0.00077 mgm.

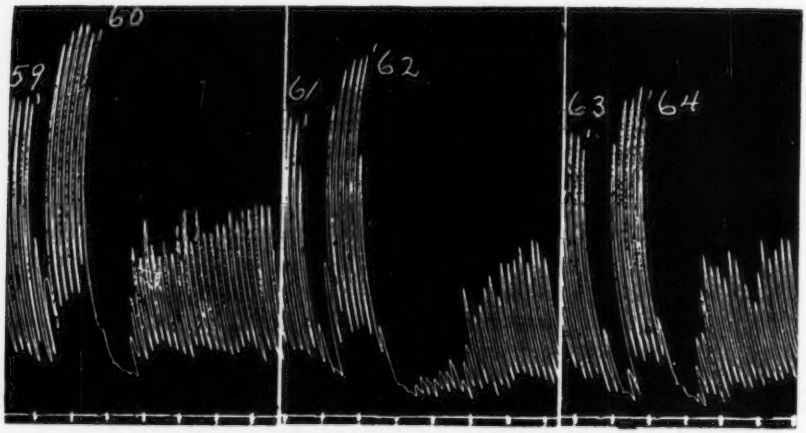

Fig. 4. Intestine tracings; bloods from cat 808. At *59*, *61* and *63* Ringer was replaced by indifferent blood and this at *60* by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000, at *62* by the second adrenal blood specimen and at *64* by the fourth specimen. All the bloods were diluted with 3 volumes of Ringer, the adrenalin blood after adding the adrenalin. Reduced to three-fourths.

per minute for the cat or 0.00025 mgm. per kgm. per minute. The fourth specimen, collected after isolation of the head arteries (before occlusion), was decidedly weaker than 1:2,500,000, weaker than 1:3,125,000 and probably slightly less than 1:3,750,000 (fig. 4, obs. *60* and *64*). It was assayed at 1:3,900,000, corresponding to the same output as before isolation of the arteries. The vasomotor response to occlusion of the head arteries was excellent, the blood pressure reaching a maximum of 170 mm. of mercury. The sixth specimen, collected during the occlusion, was decidedly stronger than 1:2,500,000 adrenalin, decidedly weaker than 1:1,250,000 and not very different from 1:1,875,000 (fig. 5, obs. *38* to *44*). It was assayed at 1:1,875,000, corresponding to an output of 0.0023 mgm. per minute for the cat or 0.00075 mgm. per kgm. per minute.

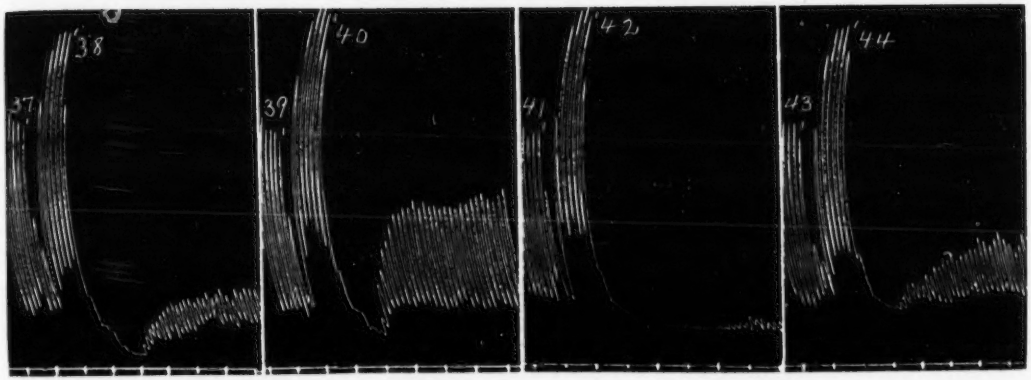

Fig. 5. Intestine tracings; bloods from cat 808. At *37*, *39*, *41* and *43* Ringer was replaced by indifferent blood and this at *38* by the sixth adrenal blood specimen, at *40* by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000, at *42* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000 and at *44* by indifferent blood to which was added adrenalin to make a concentration of 1:1,875,000. All the bloods were diluted with 3 volumes of Ringer, the adrenalin bloods after adding the adrenalin. Reduced to three-fifths.

Out of four experiments in which adrenal blood was obtained before and after isolation of the head arteries one yielded a low epinephrin output before occlusion of the arteries. No difference was found in the epinephrin output before and after isolating the head arteries. During the occlusion in this animal (cat 809), the blood pressure effect yielded a "double" or "dissociated" curve. The epinephrin output at this time was markedly increased. The bulbar centers did not recover after releasing the arteries and a half hour later, when the blood pressure was only a little above 40 mm. of mercury, the epinephrin output was reduced to about one-third the initial rate.

Condensed protocol. Cat 809; male; weight 2.68 kgm.

Under ether anesthesia, inserted tracheal and carotide annulae and prepared cava pocket.

9:18 a.m.; first adrenal blood specimen, 1.6 grams in 30 seconds (3.2 grams per minute)

9:18½ a.m.; second specimen, 4.9 grams in 2 minutes (2.45 grams per minute)

9:25 to 9:50 a.m.; prepared head arteries for occlusion

10:00 a.m.; blood pressure, 115 mm. Hg

10:01½ a.m.; third specimen, 1.5 grams in 30 seconds (3.0 grams per minute)

10:02 a.m.; fourth specimen, 4.55 grams in 2 minutes (2.28 grams per minute)

10:10 a.m.; blood pressure 91 mm.; occluded head arteries

10:10½ a.m.; fifth specimen, 1.7 grams in 45 seconds (2.3 grams per minute)

10:11¼ a.m.; sixth specimen, 5.0 grams in 2 minutes (2.5 grams per minute)

10:15½ a.m.; released head arteries; blood pressure after release, 60 mm. Hg

10:45 a.m.; blood pressure 45 mm. Hg

10:45¾ a.m.; seventh specimen, 0.5 gram in 30 seconds (1.0 gram per minute)

10:46½ a.m.: eighth specimen, 2.95 grams in 5 minutes (0.6 gram per minute)

10:57 a.m.; blood pressure 42 mm. Hg; occlusion of head arteries yielded no response. Obtained indifferent blood.

The second adrenal blood specimen collected before and the fourth specimen after isolating the head arteries (before occlusion) gave identical reactions. They were decidedly weaker than 1 :5,000,000 adrenalin, probably slightly weaker than 1:7,500,000 (fig. 6, obs. *56* to *60*) and decidedly stronger than 1:12,500,000. They were assayed at 1:8,000,000, corresponding to an output of 0.0003 mgm. per minute for the cat or 0.00011 mgm. per kgm. per minute in both cases, since the blood flows were practically the same. The sixth specimen, collected during occlusion of the head arteries, was decidedly stronger than 1:2,500,000 adrenalin, stronger than 1:1,250,000 and weaker than 1:625,000 (fig. 7). It was assayed at 1:850,000, corresponding to an output of 0.003 mgm. per minute for the cat or 0.001 mgm. per kgm. per minute. The eighth specimen collected a half-hour after releasing the head arteries had a very low concentration of epinephrin in spite of the small blood flow. The blood pressure had fallen to 42 mm. of mercury, and the bulbar centers did not respond to occlusion of the head arteries at this time. A low

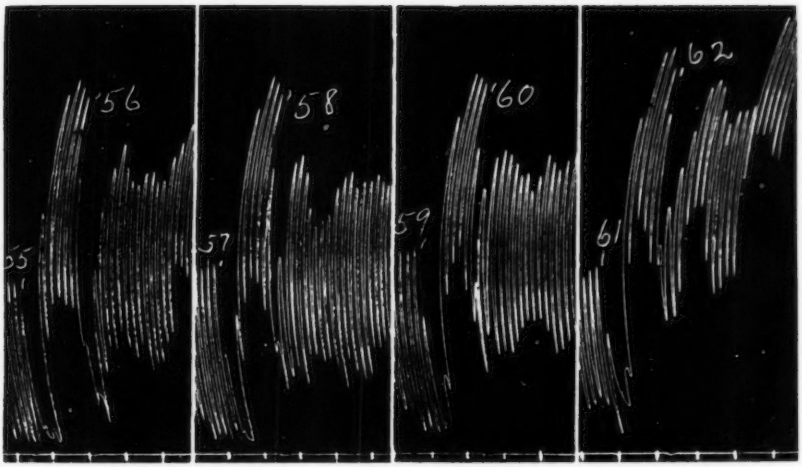

Fig. 6. Intestine tracings; bloods from cat 809. At *55*, *57*, *59* and *61* Ringer was replaced by indifferent blood and this at *56* by indifferent blood to which was added adrenalin to make a concentration of 1:7,500,000, at *58* by the fourth adrenal blood specimen, at *60* by the second specimen and at *62* by the eighth specimen diluted with one volume of indifferent blood. All the bloods were diluted with 3 volumes of Ringer, the adrenalin blood after adding the adrenalin and the eighth specimen after adding the indifferent blood. Reduced to three-fourths.

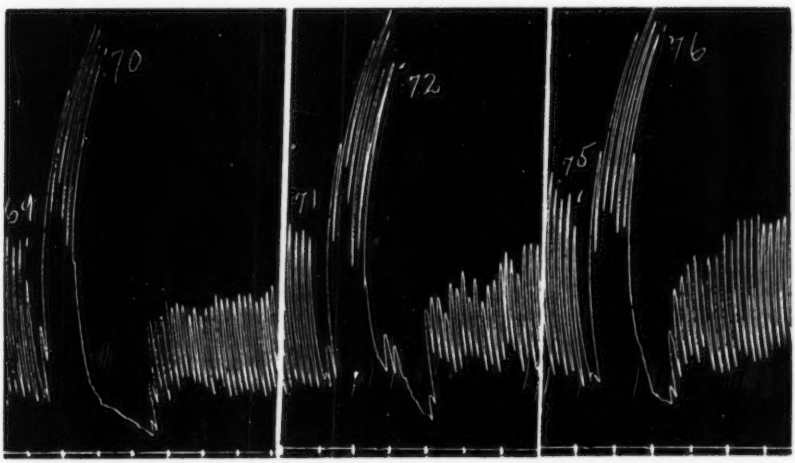

Fig. 7. Intestine tracings; bloods from cat 809. At *69*, *71* and *75* Ringer was replaced by indifferent blood and this at *70* by indifferent blood to which was added adrenalin to make a concentration of 1:625,000, at *72* by the sixth adrenal blood specimen and at *76* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with 3 volumes of Ringer, the adrenalin bloods after adding the adrenalin. Reduced to three-fourths.

epinephrin concentration in adrenal blood collected with small blood flows has been seen when the conditions of the experiment resulted in interference with the nervous paths concerned with epinephrin secretion. Examples of such conditions are afforded by the blocking of efferent paths to the adrenals by nicotine, or the spinal shock that may be associated with acute cord transections. Indifferent blood was added to the eighth specimen before assaying it, since a low epinephrin concentration was not expected with the slow blood flow. Diluted with one volume of indifferent blood it gave a weaker reaction than 1:7,500,000 (fig. 6, obs. *56* and *62*), somewhat stronger than 1:15,000,000, and not far from 1:12,500,000. The dilution was assayed at 1:12,000,000, i.e., 1:6,000,000 in the undiluted blood, corresponding to an output of 0.0001 mgm. per minute for the cat or 0.000037 mgm. per kgm. per minute.

The next experiment (cat 810) affords another example of marked reduction of the epinephrin output, after occlusion of the head arteries, when the resulting complete anemia of the upper part of the central nervous system has caused the blood pressure to fall quickly to spinal level. During the isolation of the arteries, in this animal, repeated traction on the arteries was purposely made to see what effect this may have on the epinephrin output during collection of the adrenal blood specimen taken before occlusion of the arteries.

Condensed protocol. Cat 810; male; weight 2.0 kgm.

Under ether anesthesia, inserted tracheal and carotid cannulae and prepared cava pocket.

9:10½ a.m.; first adrenal blood specimen, 1.05 grams in 30 seconds (2.1 grams per minute)

9:11 a.m.; second specimen, 3.85 grams in 2 minutes (1.9 grams per minute)

9:15 to 9:35 a.m.; prepared head arteries for occlusion; (purposely caused intermittent short anemias by traction on ligatures)

9:38 a.m.; third specimen, 1.7 grams in 30 seconds (3.4 grams per minute)

9:38½ a.m.; fourth specimen, 5.9 grams in 2 minutes (2.9 grams per minute)

9:55 a.m.; blood pressure, 65 mm. Hg; occluded head arteries

9:55½ a.m.; fifth specimen, 0.5 gram in 30 seconds (1.0 gram per minute)

9:56 a.m.; sixth specimen, 0.85 gram in 6 minutes (0.14 gram per minute)

Blood pressure at end of collection, 46 mm. Hg. Obtained indifferent blood.

The second adrenal blood specimen, collected before isolating the head arteries, was decidedly weaker than 1:3,125,000 adrenalin, somewhat weaker than 1:3,750,000 (fig. 8, obs. *26* to *30*), and stronger than 1:5,000,000. It was assayed at 1:4,000,000, corresponding to an output of 0.00047 mgm. per minute for the cat or 0.00024 mgm. per kgm. per minute. The fourth specimen, collected after securing the head arteries and moderate intermittent traction of the vessels by pulling on the loose ligatures around them, was somewhat weaker than the second (fig. 8. obs. *24* and *26*). It was decidedly weaker than 1:3,750,000, (fig. 8,

ob. *28*), slightly weaker than 1:4,375,000 and slightly stronger than 1:5,000,000. It was assayed at 1:4,500,000, corresponding to an output of 0.00064 mgm. per minute for the cat or 0.00032 mgm. per kgm. per

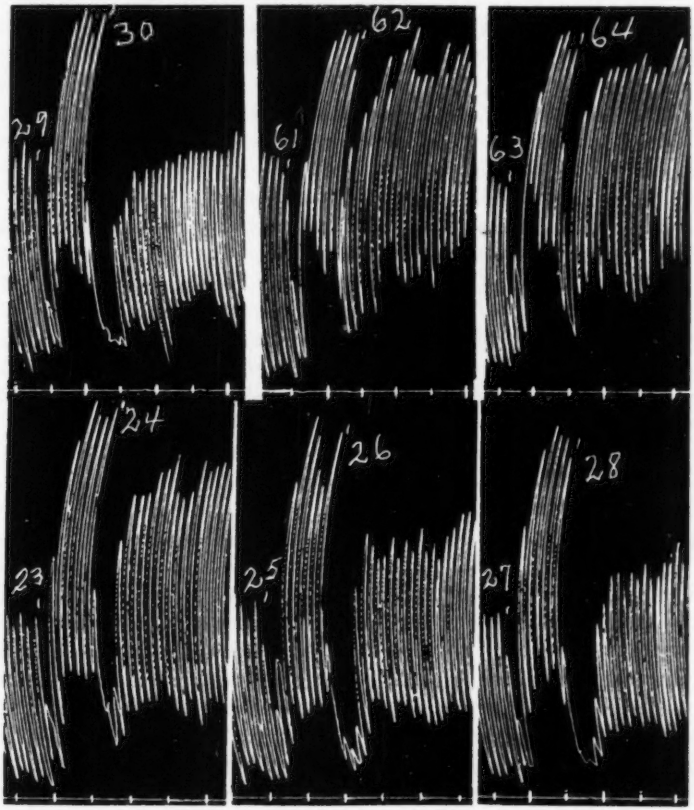

Fig. 8. Intestine tracings; bloods from cat 810. At *23*, *25*, *27*, *29*, *61* and *63* Ringer was replaced by indifferent blood and this at *24* by the fourth adrenal blood specimen, at *26* by the second specimen, at *28* by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000, at *30* by indifferent blood to which was added adrenalin to make a concentration of 1:3,125,000, at *62* by indifferent blood to which was added adrenalin to make a concentration of 1:15,000,000 and at *64* by the sixth specimen diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes of Ringer, the adrenalin bloods after adding the adrenalin and the sixth specimen after adding the indifferent blood. Reduced to four-fifths.

minute. Occlusion of the head arteries caused only an insignificant rise of blood pressure (from 65 mm. to a maximum of 86 mm.) which fell rapidly to 44 mm. of mercury. The sixth specimen, collected during the

occlusion was obtained when the blood flow through the adrenals was so slow that only a small amount of blood was gotten. This was unfortunate as it did not permit the assay of the specimen without adding indifferent blood, and with such a low concentration it could have been better assayed undiluted. In spite of the very slow flow the concentration was relatively low. Diluted with three volumes of indifferent blood it was only slightly stronger than 1:15,000,000 adrenalin (fig. 8, obs. *62* and *64*), and not very different from 1:12,500,000. It was assayed at 1:12,000,000, i.e., 1:3,000,000 in the undiluted blood, corresponding to an output of 0.000047 mgm. per minute for the cat or 0.000024 mgm. per kgm. per minute, a reduction to one-tenth of the initial rate of output.

In one experiment (cat 805), after the head arteries were isolated and the cava pocket prepared, the blood pressure was 92 mm. of mercury. During the collection of adrenal blood (before occlusion of the arteries) the pressure gradually fell to 63 mm. at the end of the collection (in 4 minutes). The assay of this blood showed an output of 0.00038 mgm. per kgm. per minute. Occlusion of the head arteries caused a gradual blood pressure rise to 84 mm., falling gradually during the collection of adrenal blood to 53 mm. at the end of the collection. On releasing the head arteries the pressure fell still farther, to 39 mm. The assay showed the same output as for the preceding specimen.

From the foregoing experimental data it is evident that the epinephrin output from the adrenal glands can be augmented by occluding the head arteries for a short period. The increase in the rate of secretion occurs at the time when the bulbar centers are responding to the resulting anemia and outlasts considerably the period of bulbar excitation. While these experiments do not yield evidence on the nervous mechanism by which the increased output is brought about, it might occur either through stimulation of an epinephrin secretory mechanism in the anemic area connected with that which seems to exist in the upper dorsal cord, or through removal of an inhibitory influence from a higher center. The latter possibility is suggested by the facts that the increased rate of secretion does not often exceed the upper limit of the normal range of spontaneous epinephrin output usually obtained with the same method of assay, and that it persists long after the period of excitation of the brain and bulb. The transient diminution of epinephrin output which sometimes precedes the prolonged augmentation when strychnine is administered subcutaneously, or with small intravenous doses, also suggests the possibility of the existence of an inhibitory mechanism.

Occlusion of the head arteries induces asphyxia of the upper part of the central nervous system, and it is of course possible that total anemia, causing a very profound asphyxia locally, may thus stimulate an epinephrin-secretory mechanism. There is at present, however, no evidence

that asphyxia of the entire animal is capable of augmenting the output of epinephrin.

Gley and Quinquaud (14) found that the adrenal glands are not essential in eliciting the rise of blood pressure caused by asphyxia, since they were able to produce as good a rise after adrenalectomy as before. In another paper (15) they found that adrenal vein blood collected from a dog during asphyxia, when injected into the circulation of another dog, caused a somewhat larger rise of blood pressure than a similar quantity of adrenal blood collected without asphyxia. They interpreted this as indicating an increased secretion of epinephrin, although they state the output is not great enough to cause physiological effects.

The conclusion than an increased epinephrin output from the adrenals is induced by asphyxia cannot be made from their experiment. That blood collected from the adrenals during asphyxia gives a greater blood pressure rise, when injected into another animal, than a like quantity of adrenal blood without asphyxia could only indicate a greater concentration of epinephrin in the asphyxia specimen. If the rate of blood flow through the adrenals is diminished during asphyxia, a greater concentration of epinephrin would be present in the adrenal blood if the rate of output remained the same. Without knowing the rate of blood flow through the glands, the rate of epinephrin secretion cannot be determined, even if a quantitative determination of the concentration is made. It was found by Stewart and Rogoff (16) that when the concentration of epinephrin in the adrenal vein blood of a dog increased during prolonged asphyxia (Gley and Quinquaud caused asphyxia for 2 to 4 minutes), the blood flow declined correspondingly and the output of epinephrin remained unchanged.

Cannon and Hoskins (17) collected blood, through a catheter, from the vena cava near the orifices of the adrenal veins and tested it on an isolated segment of rabbits' intestine. They reported an increased epinephrin output during asphyxia. In their specimens of mixed blood the adrenal contribution could not be known. Nor could they determine the epinephrin output in this way even if the epinephrin concentration in the blood had been measured (which they did not do), since they could not know the rate of blood flow through the glands at the time the specimen was obtained.

At present there is no evidence that asphyxia is capable of increasing the rate of epinephrin secretion from the adrenals. The most direct way in which this can be determined is to collect the blood from the glands without and during asphyxia, determine the rate of blood flow during the collection of the specimens and properly estimate the epinephrin concentration in the blood. When tested in this manner, Stewart and Rogoff (18) found no increase in the epinephrin output during asphyxia.

Kodama (19), using the intestine segment method, has stated that he has obtained evidence that asphyxia increases the epinephrin output. As a matter of fact, even on the basis of the numbers given by him he produces no real proof of his statement, but rather the contrary. He gives six single observations on the effect of asphyxia, each intercalated in a series of observations on the effect of sensory stimulation. Even if his determinations of the output could be accepted we should consider nearly all of these as negative, either because the output during asphyxia did not clearly exceed the initial output, or because it did not clearly exceed the output during the preceding or subsequent period without asphyxia. What, for instance, is the use of quoting an output given as 0.00065 mgm. per kgm. per minute in a cat during asphyxia as an example of an increase produced by asphyxia, when in the same experiment the initial output (without asphyxia) was 0.00054 mgm.? In another experiment the output during asphyxia for 60 seconds in a cat is given as 0.00190 mgm. per kgm. per minute, whereas in the subsequent period of 90 seconds without asphyxia the output is given as 0.00177 mgm. Differences of this order cannot, in general, be estimated by this method. In another experiment, on a dog, the output during asphyxia is given as 0.00043 mgm. per kgm. per minute, the output at the beginning of the experiment (without asphyxia) being 0.00044 mgm.

In a cat the output during asphyxia is given as 0.00442 mgm. per kgm. per minute, but this is less than the output in the preceding period without asphyxia, 0.00580 mgm. per kgm. per minute. The same experiment is also used to prove that sensory stimulation increases the output, although much the highest output during the experiment was the one just referred to (25 times the average determined by Stewart and Rogoff (20)), and this was obtained for a specimen collected without stimulation (and without asphyxia).

Unfortunately it is only too clear from Doctor Kodama's paper that he has not adequately mastered the method of assay, and that his results for this reason possess no value. We say this with regret as he has evidently spent much time on the research. One of the best proofs that he does not know how to make the assay is the excessive outputs obtained by him. Not only are his average initial outputs, before any stimulation of nerves or asphyxia, two to three times as great as ours for cats and four to five times as great for dogs, but in particular experiments the outputs, both without and with stimulation, rise to what we are obliged to call fantastic heights, and the same, of course, for the concentrations. Thus, in a dog the initial output is given as 0.00326 mgm. per kgm. per minute, about 15 times the average found by us in dogs. The concentration for the various specimens ranged from 1:330,000 to 1:70,000. It was 1:100,000 for a specimen collected without stimulation, although the adrenal blood flows were satisfactory.

There is no real evidence in his paper that the blood specimens contained such concentrations or that in the way in which the method was employed they could have been properly assayed, if they did exist. The total quantities of epinephrin which according to his data must have been given off during some of his experiments are also incredibly high. For example, in the experiment on the dog just referred to, in the 9½ minutes for which blood was collected 0.24 mgm. epinephrin must have been given off according to the outputs quoted, that is one-fourth of the total store found at the end of the experiment. As the author considers that the epinephrin store at the end had not suffered any notable depletion, epinephrin must have been formed at such a rate that the whole store of the adrenals was being replaced in about 38 minutes. During the rest of the experiment (91 minutes), while the preliminary operations were being carried out, etc., it must be supposed that epinephrin was being given off to the blood. If this was occurring at about the rate measured in the

first observation (0.00326 mgm. per kgm. per minute), 2.38 mgm. must have been discharged in the 100 minutes for which the experiment lasted. This is 2½ times the total store found at the end of the experiment in the glands, and an amount of epinephrin must have been formed and discharged in 36 minutes equal to the total store. Apparently this does not strike Doctor Kodama as at all unlikely, as he dwells with complacency on his prodigious outputs and concentrations.

If the total blood in this dog was 500 cc. (the animal weighed 7.2 kgm.) and the minute volume of the heart 1000 cc., the 6.9 cc. of blood given as the flow per minute through the adrenals at the beginning of the experiment, with a concentration of 1:300,000, would keep up in the whole of the arterial blood a concentration of nearly 1:40,000,000, rising during stimulation of the median nerve to twice this concentration. This is on the assumption that no epinephrin is destroyed in the pulmonary circulation. If such concentrations were present in the carotid blood they could be detected easily with moderately sensitive intestine segments. During nerve stimulation the output is stated to have risen to 35 times the average output found by us in dogs. The concentration of epinephrin in the plasma of the adrenal vein blood at this time must probably have been not less than 1:40,000. All through the paper one is struck with the great concentrations, even with relatively good adrenal blood flows, and even when nerves were not stimulated. On the average in the cats the concentration in the initial specimen, before stimulation of nerves had been begun, is about 1:800,000, and in dogs about 1:1,000,000, far higher values than those obtained by us.

This is not the place to enter further into detail in regard to this matter. Conclusions drawn from such results as regards the effects of sensory stimulation or asphyxia on the epinephrin output can only be regarded as guesses.

The rabbit intestine segment method, introduced by Stewart (21) and independently by Hoskins (22), is, although not properly employed by Kodama, "unambiguous," as we have claimed, since it depends upon the assay of the epinephrin concentration by a reaction, which for practical purposes is given only by epinephrin in the blood. The method of estimating the rate of epinephrin output used by us is "correct in principle," since it joins to a determination of the epinephrin concentration by the intestine segment a determination of the rate of blood flow from the adrenals. The question of the factors which influence and sustain the epinephrin output has nothing to do with the reliability of the method, which estimates the output at a given time without reference to the way in which it is produced. The frog perfusion method is we think not "unambiguous," because the vasoconstrictor effect of the serum cannot easily be eliminated, if at all. The blood pressure and denervated eye reactions, in the animal whose epinephrin output is being estimated (by "auto-assay"), are not ambiguous if adrenal blood collected in a cava pocket is allowed to act without other changes in the conditions, but are so ambiguous as to be useless if attempts are made to employ them while the conditions of the test objects are being greatly changed, as by sensory stimulation, asphyxia, etc.

SUMMARY

A majority of the cats showed a definite increase in the rate of epinephrin output from the adrenals at the time when cerebral anemia was producing the usual vasomotor and other responses. Rarely did the maximum output exceed the upper limit found in cats under our ordinary experimental conditions without cerebral anemia. An increase in the output persisted considerably beyond the period of bulbar excitation.

That the increased rate of epinephrin secretion is not responsible for the vasomotor phenomena associated with occlusion of the cerebral arteries is evident from the fact that at the time when the typical blood pressure changes were taking place the adrenal epinephrin was not passing into the circulation but was being collected through a cannula.

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THE EFFECTS OF EXPOSURE TO HIGH TEMPERATURES UPON THE CIRCULATION IN MAN[1]

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The following experiments are a part of a study of the physiological effects upon man of temperature, humidity and air motion, which is being conducted coöperatively by the Bureau of Mines, the U. S. Public Health Service and the American Society of Heating and Ventilating Engineers. The Bureau of Mines is coöperating because of the bearing of this study on the ventilation of deep warm mine workings.

Object. Numerous studies on the regulation of body temperature in man have elucidated the thermal properties of the organism. Few data are available, however, upon the nature of the regulation of bodily functions in extreme atmospheric conditions. Pioneer studies by Haldane (1) and Sutton (2) indicate the importance of respiratory factors in the body's incompetency to regulate accurately under such conditions, while the preliminary studies by Boycott and Haldane (3), Sayers and Harrington (4) and others indicate that momentous changes take place in the circulatory system. Alterations in muscular condition that occur at such times (5) may possibly result from the circulatory changes, by analogy to the conditions which prevail in exercise as outlined by Bainbridge (6).

The following experiments were designed to investigate the occurrence and nature of the changes in the circulation, and the "heat stroke," which are the most obvious effects of exposure to high temperatures. In the extended experiments upon the physiological effects of various environmental temperatures at the Pittsburgh Experiment Station (7), (8), accurately standardized conditions of temperature, humidity and air motion have been obtained. In this paper only those atmospheric conditions are considered which cannot be endured by man for an indefinite length of time.

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Plan of tests. Some data from an extended series of experiments upon some 20 different men were available preliminary to the present study. The acid-base equilibrium was studied upon two subjects (E. F. A. and W. B. F.) in special experiments. Both were accustomed to exposure to high temperatures and humidities. The atmospheric conditions used in the seven special tests were either 39.5°C. or 40.7°C., with 100 per cent relative humidity and no air motion. Such conditions could be endured for approximately one hour. The constant-temperature or psychrometric room and its equipment has been described elsewhere (7), (11); in every experiment the atmospheric conditions were maintained within 0.3°C. throughout the test. In the special tests blood samples were collected five minutes before entering the psychrometric room and two minutes after leaving it. Other observations were made at irregular intervals.

Data and methods. The physiological data obtained in the study of this problem were as follows:

a. The pulse rate, respiratory rate, blood pressure, body weight loss and respiratory metabolic rate were determined by the usual methods; the technique used in this laboratory has been described already (7).

b. Alveolar carbon dioxide tensions were measured by the method of Haldane and Priestley (9), (10).

c. The hydrogen ion concentration was measured by the recent method of Cullen (12) upon 1 cc. of plasma from the venous blood collected under rigorous precautions to prevent the loss or accumulation of carbon dioxide.

d. The bicarbonate concentration of blood was determined upon 1 cc. of the same plasma according to the modified method of Van Slyke (13), (14).

e. The carbon dioxide dissociation curve was determined by equilibrating at 37°C. a sample of defibrinated blood with various tensions of carbon dioxide. The precautions outlined by Christiansen, Douglas and Haldane (15) were observed carefully. The carbon dioxide taken up by the blood was determined in Van Slyke's (16) apparatus, liberating the gas with lactic acid and absorbing it in 20 per cent NaOH (17), and the gas tension was measured on the small gas analyzer of Haldane (10).

f. The excretion of bicarbonate in urine was followed and the concentration of the bicarbonate was measured upon the small blood-gas analyzer of Haldane (18); and comparative determinations of the urinary hydrogen ion concentration were made as in Cullen's (12) procedure for plasma.

g. Simultaneous collections of sweat were made (19), and changes in its hydrogen ion concentration were observed as for urine by comparison with the phosphate standards of Sörensen (20), using phenol red as indicator.

h. The body temperature was followed before and after the tests with ordinary rectal and mouth thermometers. During the exposure to high temperatures rectal, mouth and skin temperatures were recorded by means of thermocouples and potentiometer (21).

i. Subjective observations were recorded wherever possible. They included comfort, tingling, tetany, mental confusion, headache, faintness and subsequent fatigue.

Acknowledgments. Dr. W. J. McConnell, U. S. Public Health Service and Bureau of Mines, has furthered the experiments in every way possible, especially in arranging for the special tests; and in addition he has permitted me to study the data which he has obtained in numerous tests. Dr. R. R. Sayers, Chief Surgeon, Bureau of Mines, has created the opportunity for this work and taken a keen interest in it. To F. C. Houghten, C. P. Yagloglou and R. L. Lincoln I am indebted for the care in providing constant conditions in the psychrometric room and for making the measurements of body temperatures. Others who acted as subjects have taken a real interest in their difficult tasks.

TABLE 1

Change in hydrogen ion concentration (pH) and total carbon dioxide content of the plasma (volumes per cent) during exposures to high temperatures

SUBJECT	TEST NUMBER	pH BEFORE TEST	pH AFTER TEST	TOTAL CO_2 BEFORE TEST	TOTAL CO_2 AFTER TEST
W. B. F.	8 AS	7.34	7.38	77.8	65.7
W. B. F.	13 AS	7.43	7.38	83.5	61.2
W. B. F.	19 AS	7.42	7.48	78.2	71.2
E. F. A.	15 AS	7.32	7.39	77.5	66.1
E. F. A.	21 AS	7.40	7.48	67.6	58.9
E. F. A.	25 AS	7.37	7.53	70.8	52.7

ACID-BASE EQUILIBRIUM. *Results upon blood and excreta.* The most important factor in the control of the respiration and circulation, as currently recognized, is the balance of acids and alkalies in the blood. This is naturally the first factor to consider, therefore, in a study of circulatory changes. Comparative measurements of the hydrogen ion concentration of plasma before and at the end of exposure to high temperatures indicated that the actual alkalinity of the blood increased slightly. The data are given in table 1. For E. F. A. the increase was pH 0.07, or about four times the difference which Van Slyke (22) calculates usually exists between arterial and venous blood. In one case it appears that the blood became more acid, though this is probably a technical error, since the CO_2 content of the same sample of blood had changed in the same direction as in the other tests. Most of the figures which represent the changes in the hydrogen ion concentration of the plasma were about

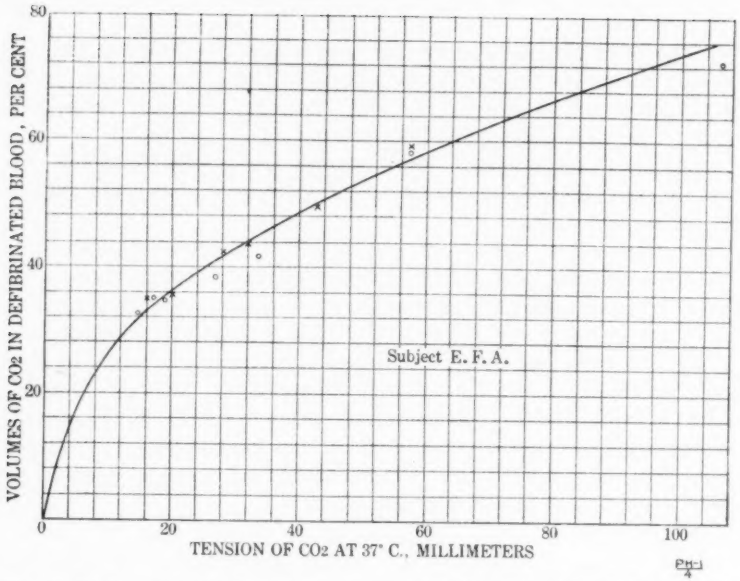

Fig. 1. Carbon dioxide dissociation curve of defibrinated blood drawn before and after exposure to high temperatures. Three experiments. Crosses, before exposure; circles, after exposure.

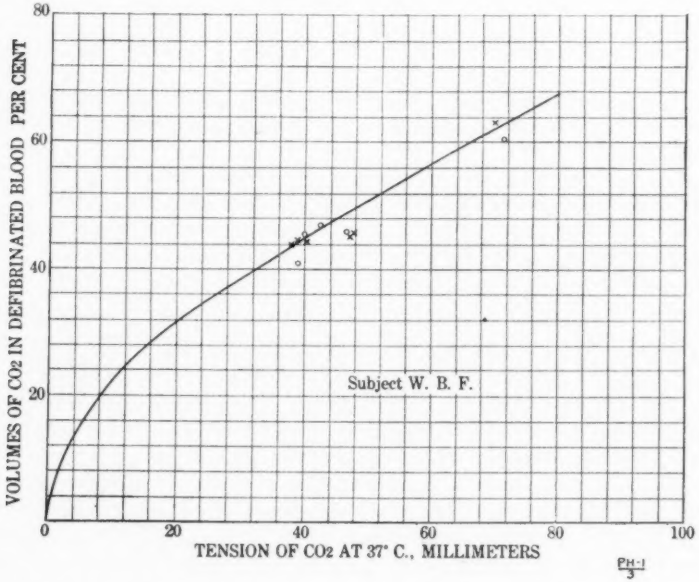

Fig. 2. Carbon dioxide dissociation curve of a second subject. Two experiments. Legend as for figure 1.

equal to the limits of error of the method as used, so that there is not complete certainty that the shift in the acid-base equilibrium is significant. The shift may be considered to be real, however, since in five determinations out of six it was in a single direction.

The bicarbonate concentration in the plasma was found always to decrease during exposure to high temperatures. This decrease, as measured by Van Slyke's titration (14), amounted to 15 per cent of the total amount in the plasma. The normal concentration varied from day to day somewhat less than ± 15 per cent.

That alkali was actually lost by the blood is indicated by the fact that in the case of E. F. A. there was always an excretion of alkali in the urine. This did not occur in the case of W. B. F. In both subjects, however, there was a decrease in the acidity of the sweat (19). Both urine and sweat are additional indicators in this respect of changes in the blood's composition.

In two experiments upon E. F. A. the bicarbonate excretion in urine has been compared with its loss from the plasma, upon the assumptions that the blood volume amounted to 3300 cc. or one-twentieth the body weight, and the plasma constituted 55 per cent of the blood. In experiment 15 AS the plasma lost 0.015 gram-molecule and 0.004 was contained in the urine. In experiment 21 AS 0.012 gram-molecule of bicarbonate was lost from the plasma and 0.006 was contained in the urine. In addition to the actual bicarbonate in the urine some alkali was accounted for, of course, by the increased alkalinity of the phosphates which it contained. The actual excretion of alkali in the sweat could not be estimated because the sweat was merely sampled from a small area of the body.

Although a considerable loss of alkali from the plasma was shown by the bicarbonate determinations, there was no significant change in the carbon dioxide dissociation curve of the blood. The data from 5 experiments are given in figures 1 and 2. In only one experiment (on E. F. A.) was there a slight decrease in the CO_2 capacity of the blood which was possibly significant. In other words, the blood was able to take up practically as much carbon dioxide after the plasma had lost some of its combining power. The result should be of wide interest in showing that bicarbonate concentration of plasma is not always an adequate measure of combining power. Apparently there was no loss of alkali from the corpuscles during the excretion of alkali from the plasma, and this accords with the known fact of the relative impermeability of corpuscles toward cations.

In one instance (W. B. F.) the actual total CO_2 content of a sample of whole venous blood was measured. The volume percentage fell from 58.5 to 47.7. Evidently the lowering was primarily due either to blowing

off of CO_2, or to the decrease in hydrogen ion concentration; for the concentration of available alkali was not diminished.

That there was neither an alkali production in the body nor a catabolism of acids, is indicated by the fact that the loss from the plasma accounted for more bicarbonate than was recovered in the urine.

During all but the first few minutes of an exposure to extreme temperatures a continuous feeling of discomfort is experienced. This is accompanied by varying sensations of tingling, particularly in the hands; and tetany and cramp of the muscles, especially in the legs, are always present. There is rarely any severe or total loss of neuromuscular control for a long period, indicating that the alkalinity is kept just at the verge of tetany.

The alveolar carbon dioxide concentration fell rapidly in all exposures to high temperature. This was early discovered by Haldane (9) and a few measurements which I have made by this method indicate that the alveolar CO_2 is almost halved during one of the present tests.

Interpretation of the results. Having reviewed the data obtained upon the acid-base equilibrium of the body during exposure to high temperatures, we can now attempt to synthesize the results. Since the actual alkalinity (pH) of the blood increased while the alveolar carbon dioxide tension decreased, it is plain that our starting point in this discussion must be with the alveolar carbon dioxide. The change in blood reaction does not account for the lowered alveolar concentration of carbon dioxide; but it is evident that a rapid loss of carbon dioxide from the blood would account for the change in reaction. Since increased ventilation by the lungs would not be caused by the observed change in reaction of the blood, which was in the alkaline direction, loss of carbon dioxide must be due to some other factor than hyperpnea which might have been excited by an increased carbon dioxide tension in the blood in the medulla.

That neither the carbon dioxide concentration nor the hydrogen ion concentration in the blood is influenced significantly by the direct application of temperature change, has been shown by others. L. J. Henderson (24) measured the change in dissociation of phosphates with temperature, and his data show that it is not significant over the range from 37° to 40°C. Stillman, Van Slyke, Cullen and Fitz (25) have shown that the bicarbonate-carbonic acid equilibrium in plasma is not affected by temperature. Haggard (26) has shown that the CO_2 dissociation curve of dog's blood is the same at all temperatures, taking the CO_2 in solution as the equilibrium concentration; and that human blood taken from the body at 40°C., a temperature obtained by immersion in a hot bath, takes up as much CO_2 at 40 mm. tension as the blood drawn at 37°C. The variation in solubility of CO_2 at these temperatures is also relatively insignificant. Similar studies of the effects of exposure to

high atmospheric temperatures upon the CO_2 dissociation curve of blood have been recently published by Flinn and Scott (59) and by Cajori, Crouter and Pemberton (60).

It appears, therefore, that during exposure to high temperatures there is a loss of CO_2 from the blood. The other changes are then found to be exactly those found in the lowering of CO_2 in the blood caused by voluntary forced breathing; the results of such experiments have been described by Cellip and Backus (28), Grant and Goldman (29), Davies, Haldane and Kennaway (30) and others. These changes are: increase in the blood's actual alkalinity, excretion of bicarbonate, diuresis, tetany and tingling. All these effects have been experienced in most of the tests which we have carried out. In the case of W. B. F., however, the acid-base equilibrium of the blood is affected less than in E. F. A., his ability to regulate this factor being greater. It may be also noted that he is able to withstand exposure to high temperatures longer than are most other subjects.

There are apparently two paths by which CO_2 is lost from the body faster than normal regulation would allow. Some hyperpnea may occur, though we have not found it a constant occurrence. That which is commonly described as heat dyspnea (1), (27) seems to be voluntary in many cases, or due to incidental exercise.

The lungs are not the only channels for the loss of CO_2 from the body. Extreme conditions of exposure always arouse sweating and dilatation of the skin capillaries. Under such conditions CO_2 may escape in the sweat, and probably to some extent through the layers of the skin. There is no direct evidence in the present work that CO_2 escapes in these ways, but the CO_2 excretion through the skin has been measured by Schierbeck (31), Wilbrand (32), (33) and others. Calculations from their data would indicate that the loss of CO_2 through the skin is exceedingly large. Moreover, the sweat is always more acid than the blood (19), even after prolonged exposures, so that all losses through the skin leave a balance of alkali remaining in the blood.

The study of the reactions of the body to high temperatures has been carried out by a number of workers upon subjects immersed in hot baths (Haggard (26), Hill and Flack (34), Bazett and Haldane (35)). Several complications arise in such experiments, from which the present ones are free. Thus it has been suggested that the over-breathing was aroused through the greater effort of respiration due to external pressure on the thorax. But the alkalosis and all the accompanying symptoms found in hot baths prevailed in the experiments in hot and humid air, where no unusual thoracic pressure occurred.

The acid-base derangement in high temperatures is an "uncompensated CO_2 deficit" followed by a "compensating CO_2 deficit." These

are areas 2 and 3 of Van Slyke's plan (22). Such derangement is produced experimentally by all forms of voluntary or involuntary forced breathing.

It is known in general that several influences vary the ordinary control of the breathing by the CO_2 tension in the medulla. These are (9), (36): high temperatures, decreased atmospheric oxygen pressure, drugs of various kinds, emotional states, and certain pathological conditions. This change of regulation is usually described by Haldane (9) and others (6) as a "hyperexcitability" of the respiratory center. It seems equally possible to consider that the regulation of breathing has come under the control of another ascendent nervous mechanism (37). Numerous nervous mechanisms may "overrule", so to speak, the respiratory regulation. They are initiated by environmental stimuli. The heat dyspnea which has been described in man is exactly analogous to the panting of the dog, although it is not known that there is any excessive ventilation in the dog at such times, since possibly only the dead space is concerned. In man the rapid breathing may be answering the "demand for cooling"; it is not completely involuntary, but when it occurs it leads to overventilation.

There are probably other factors than the intrinsic temperature of the central nervous system to initiate the acceleration of the respiratory movements when they occur. If the respiratory change were due to a hyperexcitability of the central nervous system, then the hyperexcitability would be a function of the deep or rectal temperature. The rapidity of recovery on emerging to a cool atmosphere, however, indicates that a peripheral temperature stimulus was the effective agent in modifying the respiratory regulation. This accords with the early observations of Sihler (55).

Peripheral circulation. *Arterial and capillary blood flow.* A discussion of the peripheral circulation at high temperatures must begin with the consideration of the diastolic blood pressure, because the diastolic blood pressure falls very rapidly during exposure to high temperatures. At the end of an experiment the diastolic blood pressure was very often found to be immeasurably small. The systolic blood pressure, on the other hand, is not at all parallel to the diastolic, but rises by about 20 mm. of mercury from normal. In 28 tests at "effective temperatures" (38) of 39° to 46°C. on seven different subjects, the systolic rose and the diastolic fell in all except 5 tests. This is contrary to the findings of Sayers and Harrington (4) at slightly lower temperatures, and of hot bath experiments done upon the author by Bazett and Haldane (35). In all those cases both pressures fell, though with a slight increase of pulse pressure. I interpret the present findings (7), (8) upon the blood pressure to mean that the systolic blood pressure is acting to oppose or to com-

pensate for the fall in diastolic blood pressure. The pulse is weak and flabby. The heart is working to make up for the decrease in peripheral resistance. The increased systolic blood pressure accompanies an increased pulse rate; and Douglas and Haldane (39) have demonstrated that under conditions of exercise the volume output of the heart is increased at the same time with the pulse rate. Judging from their findings, the observed increase in systolic pressure and in pulse rate together serve to augment three or four times the speed with which the blood flows from the heart.

The venous blood during exposure to high temperatures is invariably much redder than under ordinary conditions, indicating that the blood on the whole passes around the body more frequently. Although oxyhemoglobin dissociates less readily at high temperatures, it is unlikely that there is oxygen want, for holding the breath is very easy during high temperature tests, as previously shown also by Hill and Flack (34).

That the diastolic blood pressure falls so steadily suggests a failure on the part of the peripheral blood vessels to maintain their tension. The direct observations upon these vessels are comparatively few. Attempts were made to test certain of the cutaneous reactions. Everyone who has been exposed to high temperatures has seen the flushing of the skin which takes place, indicating, as Krogh (40) has proven, an immense increase in the volume of the capillaries. I have found that when the blood was quickly pressed from the capillaries of the skin, the usual color of the skin returned more rapidly during exposure to high temperatures than normally. Evidently the volume of blood in the skin capillaries is extremely large, and the capillary walls extremely relaxed.

Analysis of the vascular response. In exposures to high temperatures one may picture the response of the capillaries as follows: initially the capillaries expand, stimulated through the warm temperature sense in the skin. This increases ordinarily the blood flow through the skin (41) and therefore the total rate of circulation through the skin, and consequently the radiation of heat from the skin. The radiation outward, however, under the conditions of these experiments, was nil, since the environmental temperature and humidity were higher than that of the body. That the dilatation reflex does not concern any part of the central nervous system has been shown by various authors who obtained the response of sweating and of capillary dilatation after central connections were cut (42). The dilatation reflex is therefore of a local nature, depending entirely upon the nerves in the skin area itself. It is probably mediated for the most part by the sympathetic nervous system. The capillary dilatation thus initiated is known to spread rapidly from a local starting point until it constitutes a general cutaneous reaction (42). Apparently

the only way in which these reflexes may be stopped is by the removal of the organism from the hot environment.

Once the circulation has begun to fail, however, undoubtedly a new factor comes in, namely, the chemical control of the circulation in the capillaries, which was demonstrated by Krogh (40) and Hooker (43). Krogh believes that the pituitary secretion acts as a continual excitant to the capillaries to maintain them in a contracted condition. When the circulation slows or stops new supplies of this chemical substance fail to reach the capillaries and consequently the tone greatly decreases until the expansion of these vessels is extreme.

Capillary dilatation occurs in most of the circumstances known in which hyperpnea and acapnia are present (44). Whatever the means of coördination between breathing and vasodilatation, whether nervous or chemical, it is plain that the two reactions are commonly associated. Yandell Henderson (45) believes that lack of CO_2 in peripheral vessels leads them to dilate; the older experiments of Gaskell (46) show that increasing the alkali content of the blood decreases the blood flow in the frog; and Stewart (23) demonstrated that forced breathing is accompanied by a slower blood flow in the hands

Tachycardia. It is well known that the heart's rate increases rapidly during exposure to high temperatures. Would such an increase occur if the heart were isolated from the body? Martin (47) states that the dog's heart has a temperature coefficient (for 10°C.) of about 3 for 30° to 40°C. Our data show that the human heart in the body increases its rate 3 times for only three degrees from 37° to 40°C., so that the acceleration is far above that of the excised heart. The heart rate is fairly well correlated, nevertheless, with the heart's temperature. This correlation has been previously shown by Mansfeld (48) and others.

Evidently the reaction to high temperatures is carefully graded in its amount. There is something unexpectedly precise, and the circulatory modifications are orderly. The initial physiological effect is apparently the gradual peripheral vasodilatation, accompanied by loss of CO_2 and acapnia. Do these conditions ordinarily lead to increased heart rate?

There is no experimental evidence to show that the heart is regulated to correspond with the hydrogen ion concentration of the cells of the medulla (6). If there were such a correspondence, the regulation would be expected, in this case, to shift with the respiratory hyperexcitability, so that little or no heart acceleration would result.

The mechanism by which the rate of the heart is correlated with the expansion of the peripheral vessels in general has not been greatly elucidated so far. It is known from the work of Yandell Henderson (45) that the venous filling of the heart is a controlling factor in heart activity. We know, in other words, that the heart sometimes compensates for

lack of venous blood volume by an increase in its rate. Whether this is due to actual direct stimulation of the heart itself, or to central nervous reflexes, is not known. It is probably not due to the amount of arterial resistance; in exposure to high temperatures and in acapnia (45) the arterial resistance is apparently not lowered. It is possible that vasomotor sensory impulses are set up in the peripheral vessels which stimulate the accelerator nerves of the heart. On the whole, the circulatory failures of the shock type point to non-chemical correlation between the central and peripheral mechanism for regulating the circulation.

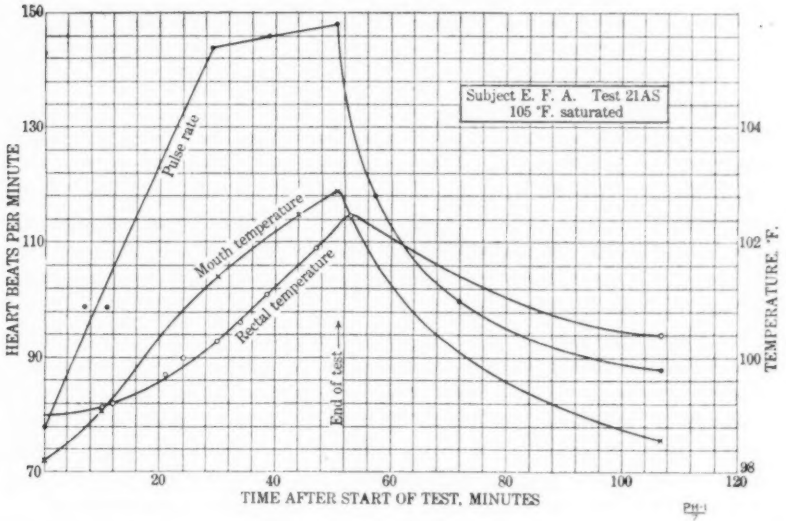

Fig. 3. Relation of pulse rate to body temperatures during and following one of the tests.

The criterion for the initiation of the tachycardia is furnished by comparing the pulse rate with body temperatures. Vernon (49) has shown that under certain circumstances the rate correlates excellently with the rectal temperatures. I have been able to find such a correlation, but only under conditions where an approach to equilibrium in temperature between the body and the environment is reached.

Correlation of the pulse-rate with the temperatures taken in the mouth, in the rectum and on the skin, show clearly that the heart rate is more nearly correlated with the mouth temperature than any other. This is evident in the protocol graphically represented in figure 3, and in the numerous ones published by McConnell and Houghten (7). The rate of rise lags slightly behind that of the mouth temperature during the first

portion of the test. After the exposure has ceased, the heart rate falls just about as rapidly as the mouth temperature, regardless of the temperature of the central organs.

We seem to have, then, a direct correlation between superficial body temperature and heart rate. The theory of Mansfeld (48) is at least wrong in that the environment to which the heart responds is not that of the pericardium, as he thought, but that of the organism's exterior.

The statement may be made, therefore, that the three primary responses to high temperatures are initiated by temperature conditions in the skin, and not by those in the central organs.

Circulatory failure. *Theories of heat stroke.* A general account of the changes in the circulation during exposures to high temperatures will be approximately as follows: Stimulation from the skin leads to reflex sweating, and sometimes to a progressive deepening and acceleration of respiratory movements. Carbon dioxide is thus lost from the blood, particularly in the sweat. The peripheral circulation greatly decreases its resistance, for an expansion of all the blood vessels occurs. It is probable that this applies equally to the capillaries and venules. As sweating goes on, the volume of circulatory blood may be significantly reduced. The heart gradually compensates for the lack of venous return of the blood by beating faster.

We have, then, in exposure to high temperatures a type of circulatory failure which would have to be included under the general category of shock. Shock is a condition in which the volume of the blood-system is increased, or the volume of blood is diminished, to the point where insufficient blood returns to the heart. When this occurs the flow of blood practically ceases, with the well-known results. This type of circulatory failure is believed by the present author to constitute "heat stroke."

Janeway and Ewing (50) have suggested that in shock the blood is mechanically prevented from returning to the heart by the increased respiration, and Henderson, Prince and Haggard (51) have confirmed this and found the same condition to result from forced breathing. As far as the author's experience goes, the shock theory of Yandell Henderson (52), (53) most nearly accounts for the type of circulatory failure found in heat stroke. In surgical shock CO_2 is lost from the blood at cut surfaces, or in the abdominal vessels which are exposed to air, or through nervous excitation to hyperpnea. In high temperatures the skin reaches a condition of extreme permeability to CO_2. The importance to the organism of a skin which does not ordinarily allow CO_2 to diffuse through it can now be realized.

Most theories of heat stroke have been concerned with the nervous system primarily. Wilbrand (54), however, suggested that circulatory failure is correlated with the reduction of blood volume by sweating, and

other investigators (37) believe that the tissues pour forth fluid at a rapid rate under conditions of high temperatures.

Test of the hypothesis. If the present view of the circulation of the blood under conditions of high temperature is tenable, then it ought to be possible to influence the circulation by means suggested by the hypothesis.

The effect of posture upon the ability to withstand high temperatures has been tested. Two tests were carried out, in which five subjects took part; the results are given in table 2.

The subjects were arranged as follows: Sitting, standing, lying down, bandaged and sitting, and doing very light work upon a bicycle ergometer.

TABLE 2

Effect of posture upon reactions to high temperatures

SUBJECT	POSTURE	BLOOD PRESSURE BEFORE TEST	TIME OF LAST BLOOD PRESSURE READING, IN MINUTES AFTER TEST BEGAN	LAST BLOOD PRESSURE READING	PULSE RATE BEFORE TEST	HIGHEST PULSE RATE RECORDED	TIME ENDURED IN TEST, IN MINUTES	BODY WEIGHT LOST, IN GRAMS
Test 21 AS—40.7°C., supersaturated								
A	Sitting	104–68	51 + 6	136–60	72	160	51	820
B	Bandaged	103–76	42	138–32	78	150	51	630
H	Ergometer	108–76	44	140–0	72	150	44	910
M	Lying	135–76	60	146–0	96	126	60	1080
S	Standing	104–66			84	132	45	740
Test 25 AS—40.7°C., supersaturated								
A	Sitting	102–68	63 + 3	134–15	72	150	63	850
B	Standing	103–65	39 + 4	132–62	72	156	39	1050
H	Bandaged	111–74	47 + 11	–39	78	136	47	620
M	Sitting	117–67	44 + 4	139–0	96	150	44	1100
S	Ergometer	101–70	23	132–64	84	120	47	620

The sitting position was the one which has been normally used in carrying out these tests, and it was independently discovered by various subjects that having the feet on a level with the rest of the body was a distinct advantage and a general relief. There was also a tendency to drop the head and to recline in the chair as soon as discomfort was experienced. The subjects who were lying down throughout the test proved to be able to stand the exposure longer than those who sat down; though this observation is inconclusive because it was found that there was slight stratification of air in the chamber (less than 1°C.). Subjects who stood up, in spite of the desire to stay in the test as long as the others, invariably had to leave first. During the test such subjects bent the head, leaned against the wall and raised the legs whenever possible. Subjects who

pedalled without any load on the ergometer also found it impossible to stay in the test very long. It was supposed that the movements would facilitate the venous circulation sufficiently to restore blood to the heart and in this way improve the condition of the subject. The position, however, seemed to be more uncomfortable and the movements did not improve the circulation. Bandaging of the arms and legs gave no improvement and no greater discomfort. Stewart (56) has shown that leg bandaging does not change the rate of blood flow in the legs.

The other test to which the hypothesis would naturally be put consists in the injection of pituitrin. Krogh (40) has shown that this is a very powerful capillary constrictor. Only two such experiments have been carried out, but with immediate and marked improvement in comfort.

Heat factor in exercise. The results obtained upon the condition of the circulatory system during exposure to high temperatures should help in the interpretation of the circulatory phenomena in exercise. In exercise we have a simultaneously increased activity of many regulatory mechanisms. Only a few of these come into play during exposure to high temperatures. In exercise, for instance, the alveolar carbon dioxide and the free alkali of the blood are greatly diminished by the production of lactic acid, as was shown by Christiansen, Douglas and Haldane (15). The exposure to high temperatures, on the other hand, does not involve an acid production of any sort, although a lowering in the alveolar carbon dioxide tension occurs. The capillary dilatation is proven by the present experiments to be independent of the appearance in the blood of lactic acid or other special substances.

Again, the exact definition of the circulatory responses to temperature enables one to decide whether the heart's acceleration in exercise is due to the heart's temperature (48) or to metabolic products of the activity (57). From the work of Martin (58) it is evident that the persistent stimulus to high heart velocity includes both factors; but the present data indicate that this temperature factor is not concerned with the temperature of the heart itself, but the temperature of the exposed skin.

CONCLUSIONS

1. Exposure to high temperatures increases the loss of carbon dioxide from the blood through the skin and lungs. This lowering of the carbon dioxide tension increases the hydrogen ion concentration of the blood and ultimately leads to an excretion of alkali from the blood. The carbon dioxide dissociation curve of the blood is not significantly altered.

2. The peripheral blood vessels are greatly dilated during exposure to high temperatures, and this dilatation continues indefinitely. The lack of a high resistance in the peripheral blood vessels prevents blood from returning to the heart.

3. The heart rate increases steadily and rapidly, and is even able to increase the systolic blood pressure. In spite of this compensating activity on the part of the heart, the blood flow back to the heart finally becomes inadequate. At this point circulatory failure or shock is complete, with faintness.

4. The rise in skin temperature seems to play the initiatory part in the control of the respiratory and circulatory reactions.

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CEREAL VALUES AS DETERMINED BY NUMBER, FERTILITY AND COMPOSITION OF EGGS[1]

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Questions frequently arise as to the relative merits of the different cereals for growth and reproduction of animals. This is particularly true relative to the value of different grains for poultry feeds and since the data for the individual grains are not very conclusive it has become the custom of some of the manufacturers of feeds, especially those intended for poultry, to add many of the most common seeds (eight to fifteen) with the hope that some of them may be beneficial at least in a supplementary way. There has been considerable work done on the value of corn (1), wheat (2), soybeans (3), barley (4), etc., in promoting the growth, especially of rats, and the evidence presented seems to indicate that certain necessary amino acids are present in grains in such small amounts as to make the abundant ones less useful. It is also generally understood that the proteins of grains are of lower biological value than those of milk or eggs but little data seem to be available as to the extent to which the grains contain amino acids capable of producing egg proteins. The relation of the quality of proteins to milk production has been studied by Hart (5) and his associates and it seemed to the writers of this article that the relation between the quality of proteins and egg production also was worthy of investigation.

Digestible nutrients poor guide of efficiency. It is not easy to evaluate, by chemical means, the differences which characterize the starches, proteins or fats of any series of grains such as barley, wheat, kafir and rye, all of which have about 80 pounds of digestible nutrients to 100 pounds of whole grain, but when they are fed to birds the response in number and composition of eggs produced bears little resemblance to the equality of the digestible nutrients fed.

In view of the above it would seem that a more accurate valuation of the special merits of different grains might be obtained by permitting the birds to decide which grains furnished the best material for egg con-

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struction. It is believed that egg building is a more critical test of the efficiency of the raw material than is the fact that it may be assimilated by the birds and used to construct or replace tissue.

Plan of experiment. It was decided to use pigeons for this test as they seem to be able to endure confinement somewhat better than chickens. A large number of mature pigeons were captured and those most healthy and vigorous were retained and mated. These were weighed, treated with sodium fluoride powder to kill lice and placed in pairs in wire cages of about 3 feet dimensions, in a warm, light room. The pigeons were all supplied with grit, oyster shell, ground bone, salt, charcoal and tap water. Each pair was given only one of the following grains: wheat, corn, oats, barley, rye, kafir, hemp, pop corn, sunflower seed, soybean seed, field peas or buckwheat.

TABLE 1

Average weights of food and feces, intake and outgo of nitrogen per day per pigeon

RATION	GRAIN INTAKE PER DAY	PER CENT OF FECES FROM GRAIN	N INTAKE PER DAY	PER CENT N EXCRETED
	grams		*gram*	
Barley	25.08	38.65	0.480	58.54
Buckwheat	33.00	27.32	0.569	49.03
Canada field peas	20.50	25.73	0.776	32.20
Corn	24.70	12.80	0.406	19.45
Hemp	30.30	20.00		
Kafir	23.80	11.15	0.425	24.47
Oats	26.20	34.79	0.476	38.23
Pop corn	28.60	7.80	0.512	26.17
Rye	26.40	34.58	0.476	55 67
Soybean	31.00	23.90	1.660	31.50
Sunflower seed	16.00	53.11	0.387	30.49
Wheat	20.72	36.25	0.393	66.15

After the birds were fed on these grains for about six months and weights, egg records, feces samples, etc., had been secured, the following changes were made. The wheat pair was shifted to the buckwheat, rye to pop corn, oats to sunflower, corn to hemp, kafir to soybeans, and barley to peas. These changes were made to eliminate individual differences in performance as much as possible. The experiment was continued three months longer on this changed diet but since the egg record and responses of the birds were quite similar to those obtained under the first plan it was not thought worth while to prolong the test. The birds were allowed to hatch eggs during both periods, but hatchable eggs were secured only from those birds receiving wheat, corn, oats and rye. The eggs from soybeans seldom had much of a shell and were often broken when laid or shortly afterwards and no hatching record was

secured from this grain. The young pigeons from wheat or rye grew to maturity and seemed to be normal but those from corn or oats only lived about a month. A record was kept of the intake of feed, as well as the per cent of feces and the nitrogen intake and excretion. These data are given in table 1.

It will be noted from table 1 that from 16 to 33 grams of grain were consumed by each bird per day and that the amount of feces produced

TABLE 2

The nitrogen distribution of the pigeon feces from various cereal grains expressed in per cent of total nitrogen of the feces

RATION	AMMONIA NITROGEN	MELANIN NITROGEN	AMINO N IN BASES	NON-AMINO N IN BASES	AMINO N IN FILTRATE OF BASES	NON-AMINO N IN FILTRATE OF BASES	TOTAL BY SUMMATION
Rye	2.40	20.80	6.80	2.80	60.62	3.38	96.80
Rye	2.28	20.60	7.20	2.40	61.16	3.42	97.06
Wheat	1.24	44.10	8.16	1.35	32.68	13.03	100.56
Wheat	1.40	43.50	8.36	1.45	33.60	12.91	101.22
Oats	0.00	21.80	9.58	1.21	63.30	2.31	98.20
Oats	0.00	21.43	9.16	1.54	64.50	0.40	97.03
Hemp	2.94	18.36	4.68	6.56	27.84	37.46	97.84
Hemp	3.13	18.01	5.00	6.20	28.40	35.66	96.40
Canada field peas	3.00	42.35	3.70	7.06	22.48	20.87	96.46
Canada field peas	2.80	43.00	4.00	6.91	22.00	21.00	96.81
Buckwheat	2.49	32.23	0.38	6.99			
Buckwheat	2.50	32.00	0.42	6.79			
Barley	0.00	32.20	3.90	3.04	55.60	16.40	101.14
Barley	0.00	22.00	4.60	2.70	52.40	18.80	100.50
Sunflower	0.00	29.10	7.74	0.96			
Sunflower	0.00	29.40	7.94	0.73			
Corn	1.27	28.53	8.76	1.18	27.52	31.21	98.47
Corn	1.32	28.40	8.20	1.65	28.00	31.13	98.70
Kafir	9.17	26.82	6.54	4.33	34.40	16.76	98.02
Kafir	9.28	27.10	6.75	4.43	35.20	16.05	98.80

from different grains varied widely. Only 7.8 per cent of feces was obtained from pop corn whereas sunflower seed produced over 53 per cent feces.

The comparatively small amount of wheat consumed daily (20 grams) and the rather high per cent of feces (36 per cent) and still higher per cent of nitrogen excreted (66 per cent) is noteworthy, especially since it was the wheat lot that laid the most eggs. It is evident that wheat proteins are remarkably well adapted to egg production and that 20 grams of wheat daily were more efficient than 26 grams of rye, 25 grams of barley or 24 grams of kafir even though they are all credited with about the same amount of "digestible nutrients."

It was thought worth while to make a study of the feces nitrogen from the various cereals, because of the wide differences in utilization of the grains by the birds.

The Van Slyke method was used in this separation, the sample first being hydrolyzed forty hours. This method may be considered of questionable value with feces samples in view of the large fiber portion, but it is believed the data will call attention to a few points which should not be overlooked in a study of this kind. The average data of duplicate samples are given in table 2.

TABLE 3

The nitrogen distribution of pigeon eggs from various cereal grains expressed in per cent of total nitrogen of the eggs

RATION	PER CENT NITROGEN	AMMONIA NITROGEN	MELANIN NITROGEN	AMINO N IN BASES	NON-AMINO N IN BASES	AMINO N IN FILTRATE OF BASES	NON-AMINO N IN FILTRATE OF BASES	TOTAL BY SUMMATION
Rye	1.39	0.00	4.06	12.66	9.92	58.84	14.12	99.60
Rye	1.25	0.00	4.00	12.20	9.70	60.00	13.10	99.00
Kafir	1.54	1.76	1.92	14.12	22.48	53.94	4.20	98.42
Kafir	1.66	1.80	1.98	14.32	22.16	53.28	5.16	98.70
Wheat	1.42	0.71	3.90	19.16	13.00	60.44	0.72	97.93
Wheat	1.48	0.81	4.20	18.60	13.52	62.04	1.38	98.55
Oat	1.28	0.18	3.22	12.06	9.80	71.40	2.00	98.66
Oat	1.20	0.20	3.32	12.34	9.80	70.60	2.74	99.00
Hemp	1.43	2.11	1.00	11.74	9.54	67.40	7.10	97.78
Hemp	1.53	2.00	1.00	11.84	9.58	66.80	7.80	99.02
Canada field peas	1.24	0.00	1.33	11.00	18.97			
Canada field peas	1.36	0.00	1.50	10.40	18.93			
Barley	1.39	2.07	0.66	14.74	17.57	50.00	1.94	98.00
Barley	1.47	1.72	0.80	14.34	18.93	58.00	2.94	97.73
Corn	1.26	0.91	3.47	11.26	12.22	70.00	3.20	101.06
Corn	1.38	1.00	3.52	11.00	12.10	70.40	2.48	100.50

It will be noted from table 2 that no ammonia could be obtained from the feces derived from oats, barley or sunflower seed; whereas in the case of kafir feces it was present in an unusually large amount. This points to a large amount of nitrogen in amide linkage in the kafir and a surprising absence of such combinations in the case of the other grains just mentioned. There was a high amino nitrogen of bases in all feces in which eggs were produced in considerable number, except in the case of sunflower seed, but other factors may account for this exception. There were great differences in both the amino and non-amino acids of the filtrates, but no interpretation of the significance of these figures will be attempted at this time.

Are eggs from different cereals of the same composition? The thought occurred to one of the writers who, after living to some extent upon eggs produced by hens feeding largely upon grasshoppers and noting their peculiar taste and the color of the yolk, wondered whether or not eggs produced from different grains differed as greatly in composition and hatchability. Accordingly fresh pigeon eggs from the different grains were hydrolyzed for forty hours, the shells being first removed, then they were treated by the Van Slyke method for the nitrogen partition. Only one egg was hydrolyzed at a time, regardless of its size. The duplicate analysis was made usually on an egg from the same bird laid several weeks after the first one. The weight of the eggs varied from 13.5 grams each in the cases of hemp or field pea eggs to 17 grams for the rye eggs. The data obtained from the hydrolyzed eggs are given in table 3.

It will be noted that there is not a wide difference in the per cent of total nitrogen in the different lots of eggs, the highest, kafir, containing 1.6 per cent and the lowest, oats, 1.24 per cent. The nitrogen distribution or character of proteins, however, was found to be quite different in the various lots. No ammonia nitrogen was found in the rye or field pea eggs whereas those from kafir, hemp and barley contained nearly 2 per cent. It is hardly to be expected that much ammonia nitrogen would be found in an egg but since so much ammonia is found in the feces from these same grains (kafir averaged over 9 per cent) high amide linkage is indicated in these feeds.

Fertility of eggs related to composition. It was hardly to be expected that there would be any great amount of melanin (tryptophane) present in the eggs, but it was surprising to find that in wheat, corn, oats and rye it averaged nearly 4 per cent while in the others only a little more than 1 per cent was found. Furthermore, it was only the eggs that had the high melanin (tryptophane) which were hatchable. It will be noted that there is a considerable portion of non-amino nitrogen in eggs, which probably originated from nucleic acids and vegetable bases. Their presence indicates that non-protein forms of nitrogen also serve a useful or at least a protein sparing purpose in the development of an egg.

Discussion. It will be noted from the data presented that when pigeons are called upon to decide what single grains are the most satisfactory for maintenance and egg production their answer seems to rate the grains in the following order of decreasing efficiency: wheat, rye, oats, corn, kafir, barley, field peas, hemp and soybeans, pop corn, buckwheat and sunflower. The last three were insufficient for maintenance of body weight and no eggs were produced from these lots. The kafir ranks alongside of rye in its egg producing properties but the eggs were abnormal in composition, and none of them were hatchable. There were usually four eggs laid instead of only two when this grain was fed.

The extent to which vitamine deficiency played a part in this experiment is not known but it is thought since the birds were mature and most of them gained in weight and laid eggs, there probably was no serious deficiency in this respect. Sunlight, too, did not seem to be a deciding factor as none of the birds were in position for the sunlight to shine directly upon the cages and two of the lots which laid the most fertile eggs were the fartherest from the windows.

It is a matter of interest to know that eggs differ materially in composition according to the source of food supplied the laying hen, and it is probable that an egg of the composition of the wheat egg, with high tryptophane and low amide nitrogen is more nutritious than a kafir egg having the opposite qualities. The difference in egg composition is important from the fertility standpoint also as it would hardly be supposed that eggs from one mixed lot of grains would nourish the developing embryo equally well with any other lot of feed having widely different composition. The evidence presented indicates that eggs from wheat, corn, rye and oats, containing high melanin (tryptophane) are hatchable and eggs obtained from the other lots are not, while pop corn, buckwheat and sunflower seed were of such a composition that no egg production was possible.

SUMMARY

There is a remarkable difference in the composition of eggs derived from different grains as well as in the capacity of different grains to produce eggs.

The egg producing power of different grains seems to rank in the following decreasing order of efficiency: wheat, rye, oats, corn, kafir, barley, field peas, hemp and soybeans, whereas no eggs were obtainable from popcorn, sunflower seed or buckwheat.

It would seem from the composition of the eggs that both nucleic acid as well as nitrogen from vegetable bases are useful in egg construction.

Only eggs from wheat, rye, oats and corn containing high melanin (tryptophane) seemed to be hatchable.

It is possible that one cause of poor hatchability of eggs is due to differences in their composition brought about by too great proportion of grains like kafir, sunflower or buckwheat.

This paper is presented as a progress report, and the writers hope that it may serve to stimulate interest of other investigators in this subject.

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EXPERIMENTS ON THE SHEEP TESTIS—CRYPTORCHIDISM, VASECTOMY AND SCROTAL INSULATION[1]

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In pursuance of sex gland studies conducted in this laboratory for the past few years, carried out largely on the rat, rabbit and guinea pig, certain important conclusions have been indicated that made desirable a study of these problems on such an animal as the sheep.

One of us has been particularly concerned with testis transplantation as well as a more exhaustive study of the problem of experimental cryptorchidism (1), (2), (3), details of which work will appear shortly,—and the other with the general problem of ligation and resection of the vas deferens (vasectomy) and its subsequent influence (4), (5).

The general results and conclusions can be summarized as follows: Testes, when removed from the scrotum of a rat or guinea pig to the peritoneal cavity, rapidly lose the germinal epithelium of the seminiferous tubules and often an apparent hypertrophy of interstitial cells occurs. Testes after retention sufficiently long to bring about germinal epithelium destruction will, if replaced in the scrotum, return to a normal condition. Vasectomy, of itself, does not result in germinal epithelium destruction and interstitial cell hypertrophy, as is so often reported in late and current literature. The majority, if not all of the recent reports to this effect have come from work on animals in which the inguinal canals are patent. Under these conditions it is very easy for adhesions to follow resection of the vas, causing retention of the testes, either in the upper part of the inguinal canal or in the peritoneal cavity. Thus, degeneration of the germinal epithelium and interstitial cell increase follows, but not as a result of vasectomy. The peritoneal retention of the testis with vas deferens, blood vessels and nerves intact will, under such conditions, be followed by germinal epithelium destruction within fifteen to twenty days; a basal layer of cells consisting of Sertoli cells and possibly some spermatogonia ordinarily remains within the tubules. Absolute correlation between the position of the testis and

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state of the tubules has been determined for fifty vasectomy cases each in rats and guinea pigs. Guinea pigs with double resection of the vas deferens have been followed for ten months and the testes found to be normal (5).

The cryptorchid experiments suggested also that in as much as normal seminiferous tubules are incompatible with an extra-scrotal position of the testis, it should follow that grafts of this organ in the scrotum would differentiate tubules containing a normal epithelium with the possible differentiation of spermatozoa. It was found that rat testis grafts in the scrotum, six months after transplantation, contained spermatozoa and all stages of spermatogenesis (2), (3). Since the testes were transplanted before the period of spermatogenesis had been established, it is absolutely certain that this degree of differentiation was accomplished within the graft itself and does not merely represent spermatozoa retained within the graft from an earlier differentiation.

This intimate relationship between the state of the seminiferous tubules and the scrotum must have a causative factor, and it is to this unknown factor that our attention has been directed. Since degeneration of the epithelium follows rapidly upon replacement of a testis within the peritoneal cavity, even without interfering with blood vessels, nerves or vas deferens, we have been led to believe that differential body temperature is a factor; infection, abnormal pressure or injury to nerve or vascular supply have apparently been eliminated as causes. The scrotal sac is a relatively exposed cavity lined by the tunica vaginalis, a derivative of peritoneum and continuous with peritoneum in those animals having patent inguinal canals; the skin layer is very thin and well supplied with sweat glands; subcutaneous fat is absent; the muscular coat is extremely thin and the vascular supply of the testis is situated to a large extent on the periphery of the gland. Moreover the size, shape and thickness of the scrotum vary with the temperature. On hot summer days the scrotum of a rat is exceedingly prominent and will protrude considerably in a post-anal position, whereas on cold days the scrotum is contracted and the testes drawn close to the body or indeed into the abdomen. This change with temperature is similar for many, if not most mammals and for man. Thus we have the physical conditions to supply a local regulator of temperature for the testes and this we believe to be the function of the scrotum. Crew (6), arguing from the findings of Benedict and Slack (7), has advanced the idea that the aspermatic condition of undescended testes may be due to a higher temperature in the abdomen. The latter authors found in man a temperature gradient of 6°C. between the internal cavities and the skin.

To test the hypothesis of a temperature factor, and to obtain additional data on cryptorchidism and vasectomy from animals that have testes

completely isolated from the peritoneal cavity—inguinal canals closed—yearling rams were obtained for experiment and the results obtained are herein described and discussed.

Three procedures were carried out: *1*, ligation and resection of the vas deferens; *2*, removal of the testis from the scrotum to the peritoneal cavity by opening temporarily the inguinal canal; and *3*, insulation of the scrotum against loss of heat by secure wrapping.

The results can be stated briefly as follows: *1*, ligation and resection of the vas deferens for 90 days does not result in degeneration of the testis;

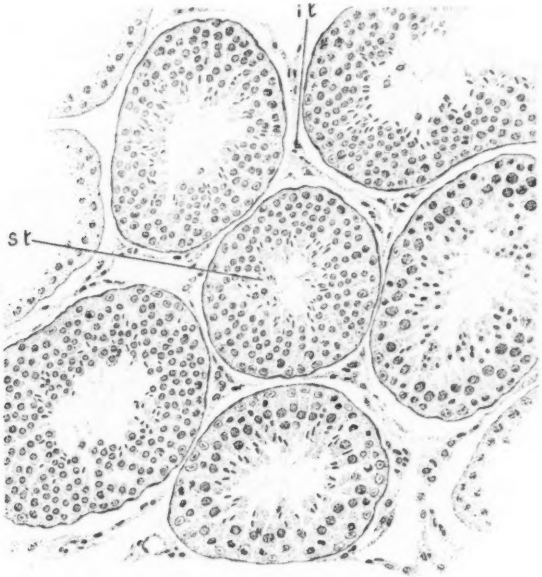

Fig. 1. Normal sheep testis (6). Control. *it*, interstitial tissue; *st*, seminiferous tubule with spermatozoa.

seminiferous tubules contain all stages of spermatogenesis. *2*, A sheep testis placed within the peritoneal cavity and surrounded by its tunica vaginalis loses the germinal epithelium and becomes a typical cryptorchid testis. *3*, Insulation of the scrotum against possible temperature loss, through secure though not too tight wrapping, produces a degenerate testis similar to the experimental cryptorchid condition.

Experiments. All of the sheep were in good flesh when received, possessed well-developed horns, grew and fattened considerably during the four months they were under observation. One, sheep 8, was somewhat smaller in size and the scrotum and testes were noticeably smaller than

the others; we judged this animal to be probably a month or more younger than the others.

Sheep 6. (Vasectomy + control.) April 21, abdomen opened by mid-ventral incision, left vas deferens ligated in two places as it crosses pelvis and before being incorporated within the spermatic cord; several millimeters of vas removed between ligatures; right testis unoperated to serve as a control for all testes. July 21, both testes removed (90 day vasectomy) and preserved for histological study. Left epididymis noticeably larger than the right. On severing the spermatic-cord an abundance of semen escaped from the left vas as if retained under considerable pressure; smears showed quantities of spermatozoa; no semen issued from the cut end of the normal vas (right).

Right testis (normal, control): Microscopically the control testis is entirely normal. All stages of spermatogenesis are readily seen and spermatozoa are numerous. Figure 1, drawn for comparison with other testes, shows not only spermato-

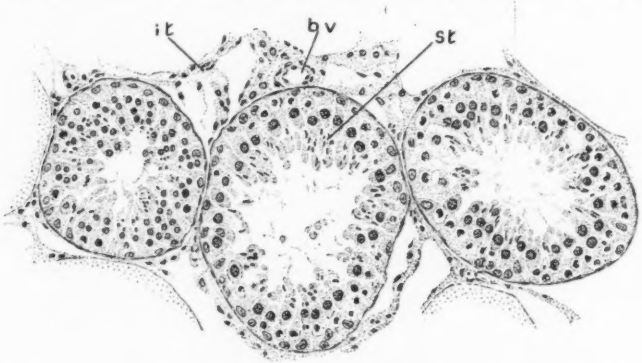

Fig. 2. Sheep testis (6) 3 months after vasectomy. *bv*, blood vessel; *it*, interstitial tissue; *st*, seminiferous tubule with spermatozoa.

genic activity but the normal intertubular tissue as well; the connective tissue is small in amount and the interstitial cells few in number. The normal sheep testis at this time of year is in full and vigorous spermatogenic activity.

Left testis (vasectomy 3 months): It should be recalled that upon removal of this testis the epididymis was noticeably larger than the control and that semen loaded with spermatozoa issued from the vas on cutting. The testis had been actively producing semen for which there was no escape excepting through the route of liquefaction and resorption by the blood or lymph stream.

Microscopically the majority of the tubules are normal and actively producing spermatozoa; tubules with an epithelium consisting of actively dividing spermatogonia, spermatocytes, and quantities of spermatozoa proves that spermatogenesis continues three months after ligation and resection of the vas deferens; figure 2 shows a few of the tubules with the attached spermatozoa. Under higher magnification numerous mitoses are seen throughout the epithelium.

Many of the tubules of this testis, particularly those situated near the straight (excurrent duct) tubules, show considerable degeneration; the lumen is either obscured or filled with debris consisting of degenerating cells and broken down frag-

ments of spermatozoa. This condition, we believe, is due to abnormal pressure within the tubules as a result of the retention of the testicular products. In figure 3 one can see a few seminiferous tubule sections and the straight tubules containing the degeneration products. It is evident, in many cases, that the contained material has been derived from sources other than the tubules in which we find it. Since the epithelium of some of the tubules is normal and reveals spermatozoa differentiated in situ still attached to the epithelium, the debris found in the lumen could not have been derived from this area and must have been transported from other regions by normal currents within the testis or by back pressure caused by the accumulations of products of other tubules. Some of the tubules have been so greatly affected that disorganization of the epithelium has occurred.

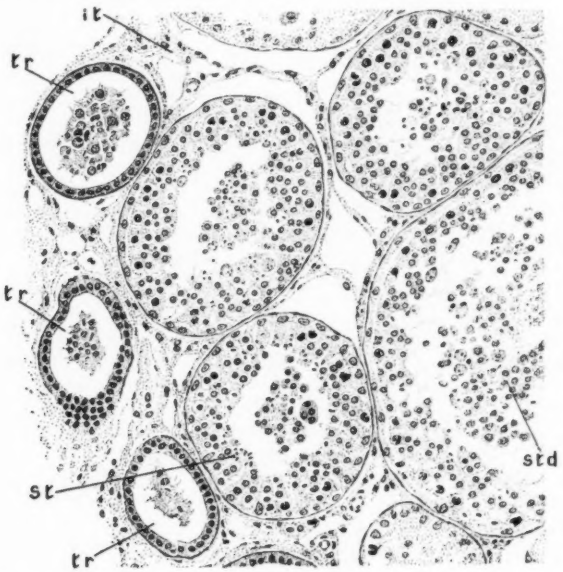

Fig. 3. Sheep testis (6) same as figure 2. *it*, interstitial tissue; *st*, seminiferous tubule with spermatozoa, containing degenerating cells; *std*, degenerating seminiferous tubule: *tr*, straight tubule containing cellular debris.

The interstitial tissue, in this testis, is similar to that of normal testes; a three month period of vas deferens ligation and resection has not operated to bring about a hypertrophy of the interstitial elements.

Sheep 8 (experimental cryptorchidism + vasectomy). May 12, right inguinal canal opened and testis, surrounded by its tunica vaginalis, pushed through opening into peritoneal cavity; spermatic cord, blood vessels, nerves and vas deferens uninjured. Left vas deferens ligated in two places as it crosses the pelvis and above its incorporation in the spermatic cord; vas severed between ligatures. Animal allowed to live seventy-six days. July 28, both testes removed and preserved for histological study.

Macroscopically the left (scrotal) testis appeared entirely normal. It was, however, somewhat smaller in size than the scrotal testes of the two other animals and it was pointed out above that both the scrotum and testes of this animal were noticeably smaller in size when the sheep were received; we judge that it was somewhat younger than the others. The left vas deferens and epididymis were not so distended as the vasectomized testis of sheep 6.

The right testis was found freely suspended in the peritoneal cavity between folds of the intestines. A few slight adhesions had formed at the point where the spermatic cord was joined to the anterior abdominal wall at the internal inguinal ring. The tunica vaginalis was intact as in the normal testis. In size this experimental cryptorchid testis was approximately one-third that of the left or scrotal testis.

Left testis: Microscopically the left testis (vasectomized) was found to be normal. Despite its comparatively smaller size for scrotal testes it was found to be entirely mature. Spermatozoa were plentiful and active mitoses in spermatogonia and

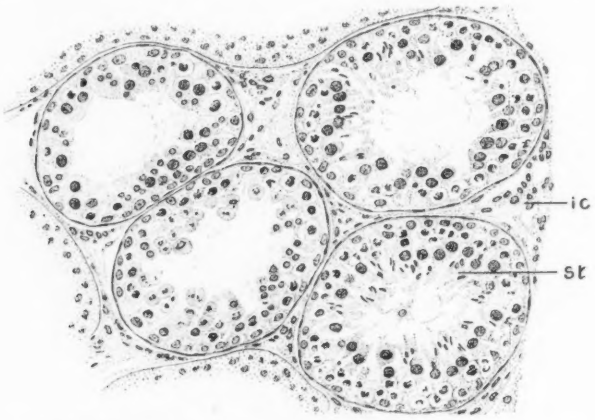

Fig. 4. Sheep testis (8) 76 days after vasectomy. *ic*, interstitial cells, *st*, seminiferous tubule with spermatozoa (active mitosis).

spermatocytes allows no other interpretation than that the testicular activity continues after ligation and resection of the vas deferens. Degenerating tubules due to accumulation of testicular products are fewer in number than in the similarly operated testis of sheep 6. Spermatogenesis appears somewhat less intense than in the latter testis. Figure 4 shows a few tubules of the testis and it not only proves that tubular activity continues in a testis seventy-six days after ligation and resection of the vas deferens but at the same time it serves as a control with which to compare the experimental cryptorchid testis of the same animal.

Right testis: Microscopically the tubules of this testis are characteristic of naturally occurring cryptorchid testes in that the germinal epithelium is to a large extent absent (see fig. 5). Within the tubules there appears a layer of cells situated next the basement membrane, often poorly stained, that have usually been called the cells of Sertoli. Despite the fact that similar layers of cells in tubules of different mammals where the testes have remained abdominal are usually so named, we are inclined to believe that spermatogonia are likewise retained within the degen-

erate epithelium. In some cases the last remains of the sloughing epithelium can be seen within the lumen but in the majority of the tubules the lumen is empty.

Interstitial cells appear to be slightly more numerous and somewhat larger in size than normal; the intertubular connective tissue on the whole is more prominent than in normal testes. This condition rather than being an example of an hypertrophy of the interstitial tissue is considered to be the result of contraction of the testis as a whole and a concentration of the intertubular tissue into a smaller space; the same amount of interstitial tissue is present that was there when the testis was normal and with the diminution in size to approximately one-third, the tissue becomes more prominent.

In this animal, therefore, vasectomy has failed to cause tubular degeneration whereas peritoneal retention of seventy-six days has produced complete tubular degeneration. In comparison with other animals we feel quite certain that approximately the same amount of degeneration would have taken place in the peritoneal testis within a period of twenty days as is present at the end of seventy-six days; degeneration to a certain extent follows peritoneal retention very rapidly whereas further degenerative changes are decidedly less rapid.

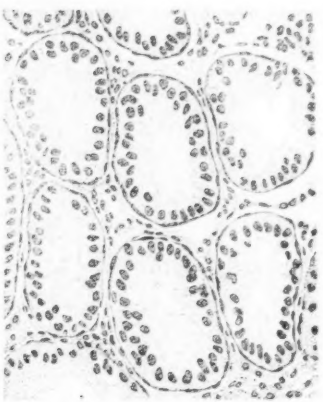

Fig. 5. Sheep testis (8). Experimental cryptorchidism 76 days. Seminiferous tubules are devoid of epithelium aside from single basement layer.

Sheep 7. (Scrotal insulation.) April 25, the scrotal sac was wrapped loosely with first a thick layer of woolen batting, second with a layer of woolen serge cloth, and finally with a water-proofed duck covering. Each separate layer was sewed to fit closely the contour of the scrotum but considerable care was taken to prevent abnormal pressure or binding of the scrotum. All layers were fitted snugly around the neck of the scrotum and underneath the body, and cloth strip supports were passed over the back and hips to prevent sagging of the coverings upon the scrotum, yet the latter could be relaxed to its full extent. July 14 (80 days after wrapping), the insulating covering was removed; one testis removed (right) and preserved for histological study. The left testis was placed within the peritoneal cavity for a period of seven days when it, too, was removed and preserved for study.

When the covering was removed at the end of eighty days the scrotum was entirely normal in shape and color. It was certain that no undue pressure could have been given by these wrappings, as a finger could easily be inserted within the innermost layer at all times.

Since we were not certain if visible effects would be produced from a scrotal insulation of eighty days' duration, and since one testis afforded an abundance of material for study of possible changes, the second testis was made cryptorchid for seven days by opening the inguinal canal and placing it within the peritoneal cavity. The left testis, therefore, was subjected to peritoneal retention for seven days in addition to the scrotal insulation of eighty days.

Right testis (scrotal insulation): Microscopically the right testis was found to have been decidedly affected by the insulation; no normal seminiferous tubules could

be found. The same extent of degeneration is not registered in all tubules but any region of the testis consists of *a*, tubules with an epithelium of approximately normal thickness *but without spermatozoa; b*, of those in which the majority of the cells of the epithelium are abnormal; and *c*, of tubules of similar structure to those found in the experimental cryptorchid testes. The degenerating tubules (see fig. 6) are of all grades from mere granulation and fragmentation of the epithelial cells, and

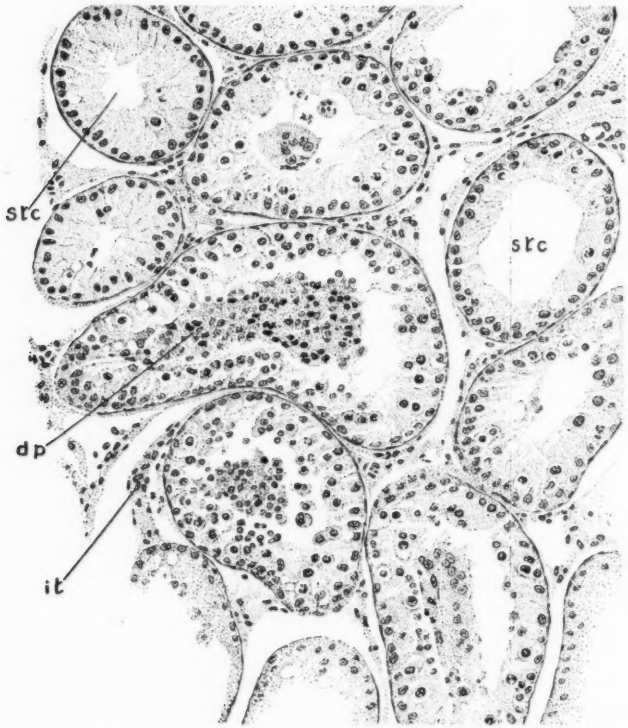

Fig. 6. Sheep testis (7). Scrotal insulation 80 days. *dp*, seminiferous tubule containing products of degenerating epithelium; *it*, interstitial tissue; *stc*, seminiferous tubules similar to cryptorchid testes.

loosening of cells from the epithelium, to almost complete disorganization of the epithelium and the filling of the lumen with the degenerating debris of cells cast out from the epithelial layer. Where destruction has gone farther the tubule contains but the remnant of the old epithelium and its single layer of cells as in the tubules of experimental cryptorchid testes. *In studying hundreds of tubules from all parts of the testis no spermatozoa have been found.*

The tubular degeneration in general is entirely similar to the progressive stages of degeneration found in testes that have been placed within the peritoneal cavity of a rat, guinea pig or rabbit. Many individual cells that have escaped from the

epithelial layer into the lumen appear normal histologically; the majority, however, are in various stages of nuclear or cytoplasmic fragmentation. Protoplasmic masses containing many nuclei, a rather typical "giant-cell" like condition, may be intermingled with granular debris in which nuclear shadows only can be seen; the approach to complete destruction is shown in masses of unorganized debris. Varying histological pictures of these degenerative changes are found in all parts of the testis. The unorganized debris in the lumen is apparently completely liquefied and absorbed, as formed materials are to a very large extent absent from the lumen of epididymal tubules. The epithelium of epididymal tubules appears not to have been affected; the ciliary lining is normal.

Left testis (scrotal insulation + cryptorchidie 7 days): Microscopically the left testis is characteristic of the experimental or normally retained peritoneal testis. A detailed description seems unnecessary since it is similar to the testis of sheep 8 that had been retained for a longer time in the peritoneal cavity.

The difference to be emphasized between this and the right testis is the absence of tubules with an epithelium of more than one cell in thickness. The destructive effects are more apparent than in the testis subjected only to the effects of scrotal insulation. It can be taken for granted that the two testes were affected to approximately the same degree by the insulation and therefore the more complete degeneration of the left testis is the result of the additive effect of seven days' residence in the peritoneal cavity.

The intertubular tissue of either testis of this animal does not appear to have been affected.

Discussion. The details of experimental work on cryptorchidism and vasectomy will be published in another place. Our chief concern at this time has to do with an interpretation of the causal influences operating to produce results that have consistently followed given procedures.

With reference to vasectomy the writers are not able to substantiate the results reported in recent and current literature that ligation or resection of the vas deferens, or both, results in the complete loss of the germinal epithelium and a hypertrophy of the interstitial tissue. We have yet to observe a single clear cut case of such degeneration that is unmistakably referable to that cause. We have performed single and double resections of the vas deferens in many animals, and one of us in particular has made observations on approximately one hundred cases of such operations in the rat and guinea pig (for detailed discussion and review of the literature on vasectomy see (5)). Double ligation and resection of the vas deferens in the guinea pig was found to be without effect at the end of ten months; each testis was normal in all respects.

Inasmuch as the majority of experiments on vasectomy have been performed upon animals having open inguinal canals (guinea pig, rat, rabbit, etc.), we are inclined to interpret the reported cases of degeneration as being due to retention (partial or complete) of the testis within the peritoneal cavity, and not from tying and severing the vas deferens. We appreciate the possible variation of reaction to the same operation in

different types of animals and we were anxious to observe the results of vasectomy on a ram.

In the sheep we can only say that ligation and resection of the vas deferens did not produce complete tubular degeneration nor did it prevent the continuance of spermatogenesis; seminiferous tubules were in pronounced spermatogenic activity three months after the operation.

It is true that some tubules, following vas deferens ligation, revealed unmistakable signs of degeneration. This was accompanied by marked distention of the epididymis and seminiferous tubules near the rete testis by testicular products and is apparently to be considered a pressure atrophy; the lumen of many tubules may contain considerable amounts of such products clearly transported from other regions and yet continue in active mitosis. What ultimate condition would follow a longer duration of such an accumulation of material is mere conjecture but it should be recalled that Shattock and Seligman (8), (9) found normal testes in the sheep one year after ligation and resection. Continued tubular activity obviously would increase the amount of testicular products contained within the lumen of the tubules and the question arises as to the fate of such products. Evidence at our disposal, obtained largely from studies on experimental cryptorchidism in other mammals, leads us to believe that the testicular products are gradually liquefied and absorbed into the circulation; in the peritoneally retained testis this proceeds much more rapidly than in a scrotal testis due apparently to higher temperatures (see below). In vasectomy operations, we believe a balance between formation of spermatozoa and their degeneration and resorption is gradually established; if production is extremely active, conceivably sufficient materials would collect to produce atrophy of the tubules in certain regions. On the other hand, if destruction and resorption were sufficiently rapid, tubular activity would remain normal. The degeneration of the tubules in the testis of sheep 8 (vasectomy 76 days) was considerably less than that in no. 6 (vasectomy 90 days). The difference, we believe, lies not in the short difference in time between the operation and removal of the gland but depends upon the differential testicular activity in the two cases; production in the latter is more rapid than in the former. Sheep 8 appeared younger, the testes and scrotum at the time of operation were smaller and mitotic activity appears somewhat less intense in the tubules. The guinea pig after vasectomy appears to have continuous spermatogenesis without destruction of the tubules by accumulated products. It is conceivable that animals with large testes in full spermatogenic activity would produce sufficient material and absorption be sufficiently low that all tubules would undergo degeneration; this condition has been approached in the ram 6. Such a complete degeneration, however, is yet to be shown.

We are not able to observe an increase of the interstitial tissue in the sheep three months after vasectomy nor in the guinea pig one year after double vasectomy, provided the testes have retained their normal scrotal relationship; if, however, adhesions or other conditions have caused inguinal or peritoneal retention of the testes, tubular degeneration may be accompanied after a time by an apparent increase in interstitial cells.

With respect to cryptorchidism and the causative factors operating to produce tubular degeneration, the writers have been inclined to follow Crew's interpretation that increased temperature is responsible. This idea carries with it the assumption that the temperature of the scrotum is somewhat less than that of the peritoneal cavity and is actively controlled perhaps by the scrotum itself; within the scrotum there should be a local temperature regulator. One of us through grafting a testis in the scrotal sac has been able to show that spermatozoa will differentiate in such a graft; thus for the first time a mammalian testis graft has possessed normal tubules (2), (3). Out of more than a hundred grafts in different parts of the animal body *spermatozoa have been found only in grafts made within the scrotal sac.* Moreover the experimental cryptorchid testes have shown us that an extra-scrotal testis does not contain normal seminiferous tubules. If the scrotum is a local regulator of temperature for the testis and this regulation is indispensable for the continuance of spermatogenesis, the testis should reveal degeneration changes if by any experimental manipulation the regulatory function of the scrotum was blocked. Acting upon this idea we have attempted to prevent the regulatory capacity of the scrotum by an external scrotal insulation against heat loss.

Of the sheep obtained for these experiments the ram possessing the best developed horns, largest scrotum and testes, was selected as the subject for scrotal insulation. The degenerate condition of the seminiferous tubules shown above is a forceful argument in favor of the supposition that the temperature-regulating mechanism has been eliminated by the insulation. We desire to emphasize the precautions taken to prevent abnormal pressure through binding the scrotum; sewing the various layers of the covering to fit the scrotal contour, the looseness of the entire set of wrappings, and the supports over the back to prevent sagging and binding, coupled with the fact that a finger could be inserted with ease inside the inner layer, give definite indications that the degenerate condition of the testis could not have been caused by undue pressure or interference with the blood supply. We believe, therefore, that a demonstration has been given that an animal will sterilize itself with its own body heat if the regulatory capacity of the scrotum is removed. We admit such an idea a little difficult to reconcile with those mammals that do not possess a scrotum. In a later publication evidence will be

presented that will aid in an understanding of these apparently different sets of conditions.

The effects of high temperatures on the testes of mammals have been reported by different investigators and one of us has for some time been carrying on experiments with the external application of higher temperatures (Moore (3) and experiments not yet reported). It is sufficient here to say that single local applications of heat to the scrotum of a guinea pig will operate to produce degeneration of the germinal epithelium of the testis. Hart (10) noted that mice retained for a month in a chamber of higher temperature than normal (36°C. to 40°C.) possess degenerate testes. Fukui (11) was able to produce degeneration of a rabbit testis by single exposures of the scrotum to the sun's heat, or to hot water or hot air heat. Steinach and Kammerer (12) report an increase in the interstitial tissue of both males and females without interfering with the activity of the germinal tissue. But aside from the work done in this laboratory Fukui alone has utilized local applications of heat to the scrotum. For an analysis of this heat phenomenon and its effect upon the testis the local application of heat is all important; animals reared in an abnormally high temperature have been found to have other organs affected than the sex glands and it is difficult to properly analyze such effects as follow this procedure.

We believe that the way has been cleared for a better understanding of the true significance of the testis behavior in transplantation, vasectomy, cryptorchidism (experimental and natural) as well as the true significance and physiology of the scrotum.

SUMMARY AND CONCLUSIONS

1. A testis of a normal ram, if removed from the scrotum to the peritoneal cavity with the tunica vaginalis intact and spermatic cord uninjured, will lose its germinal epithelium and become a typical cryptorchid testis. The germinal epithelium entirely degenerates with the exception of a single layer of cells that remain next the basement membrane (probably largely Sertoli cells).

2. The interstitial tissue of such an experimentally produced cryptorchid testis does not differ from the normal to any great extent; the Leydig cells appear to be slightly more numerous and the connective tissue a little more prominent than normal.

3. Unilateral vasectomy for 76 and 90 days does not produce complete degeneration of the seminiferous tubules and interstitial cell hypertrophy. Some tubules are degenerate, apparently from retention of the testicular products producing pressure atrophy.

4. The intertubular tissue of testes with resected vas deferens has not been visibly affected.

5. External wrapping of the ram's scrotum for 80 days results in the loss of all spermatozoa and considerable degeneration of the tubular epithelium.

6. A testis in the above degenerate condition, when removed to the peritoneal cavity for 7 days, is found to have lost the remainder of the germinal epithelium with the exception of the single layer of cells found always in undescended testes.

7. We believe that we have demonstrated that an animal can be sterilized by its own body heat and that undescended testes occurring in nature are undoubtedly degenerate because of the higher temperature to which they have been subjected.

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THE EFFECT OF RADIANT ENERGY ON THE EXCRETION OF PARENTERALLY INTRODUCED SIMPLE SALTS

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The fate of an electrolyte intravenously injected into a normal animal is twofold: excretion through the kidney and diffusion from the blood stream into the surrounding tissue. Both phases have been studied by previous workers; both may be influenced by heat or light. Van Rysselberghe (1) proved an eightfold increase in the permeability of plant cells toward electrolytes due to a rise in temperature from 0° to 30°. Lepeschkin (2) found an increased permeability due to exposure to light; and von Tröndle (3) demonstrated the independence of this "light" effect from thermal changes in the surrounding tissue. These findings are corroborated by the work of Harvey (4), J. Loeb (5), Lyon (6) and McLendon (7).

R. Gassul (8) and M. Levy (9) proved that the exposure of normal animals to various sources of radiant energy caused hyperemia of the secretory organs, independent of the penetrating power of the light emitted; Barcroft and Brodie (10) and Lamy and Mayer (11) demonstrated that an increased blood supply of the kidney and diuresis go parallel.

The experiments presented in this paper, preliminary in its character, were undertaken to determine the influence of radiant energy on the disappearance from the blood stream of intravenously injected phosphates and sulfates. Sulfates are retained for a long period of time, as has been observed by Cushny (12), Denis (13), Greenwald (14) and Sollman (15). The retention of phosphates is, in the full-grown sheep at least, less marked; in preliminary work carried out in this laboratory it was found that the phosphorus level returned to normal after a lapse of at least three hours following the administration of 26 mgm. P per kgm. in form of a 12 per cent disodiumphosphate solution adjusted to pH 7.3. If, however, a 9 per cent magnesium sulfate (crystalline) solution representing 2.4 mgm. S per kgm. was injected, twice the time was required for the disappearance of the excess and return to normal sulfur level. This marked difference in the excretory rate lead to the selection of the two salts.

The experiments were performed on full grown, fasting (16 hours) sheep; they were kept in a dark stable on a uniform diet of oats and hay. An area of 45 cm. by 40 cm. was shaved several days previous to irradiation, on the side exposed to the light source. The animal stayed in a dark room during the "dark" experiments, in bright sunlight (10 a.m. to 2 p.m.) in the "sun" experiments; two Hanovia lamps (110 volts 4 Amp.) at 65 cm. skin distance were used in the "U.V." experiment. The technique employed in the phosphorus experiments was: exposure of the external jugular vein, no anesthetic was used, drawing of a normal sample and infusion by gravity—the time required for the injection of 125 cc. was 3 minutes—of 26 mgm. P per kgm. in form of a 12 per cent disodium phosphate solution adjusted to pH 7.3; no untoward effects due to the infusion of this minimal dose, which is far below the toxic limit, have been experienced. The animal was exposed to light immediately after completed injection; 5 to 10 cc. samples were then drawn at regular intervals and the plasma analyzed according to Bell and Doisy (16); to eliminate any possible hydrolysis centrifugalization of the protein precipitate was substituted for filtration, and readings made within ten minutes; the results are mean values of three separate determinations. Standards were made up in concentration of 3, 4, 5, 6, 7, 8, 10 and 12 mgm. P per 10 cc. The experimental results are given in the form of charts where time in minutes is plotted against concentration (mgm. per 100 cc.); body (rectal) temperature is recorded by marks corresponding to the curves, i.e., circles for "sun," squares for "U.V." and crosses for "dark" experiments.

The technique for the sulfur experiment was the same, but infusion of the sulfate was preceded by a calcium chloride injection as recommended by Meltzer and Auer (17); 45 cc. of a 3 per cent calcium chloride solution were infused in the external jugular vein followed by injection of 27 mgm. S in form of a 9 per cent magnesium sulfate solution. Denis method for whole blood was used for analysis. No symptoms of toxicity, with the exception of slight dyspnea during experiment B 5 have been observed.

The data obtained in this work shows that the disappearance from the blood stream of intravenously introduced phosphates and sulfates is hastened by sunlight and ultra-violet light; this increased excretory activity is independent of fluctuation in the body temperature.

In discussing these results the possibilities mentioned in the introduction should be considered: *1*, The dilatory effect of irradiation on the peripheral vessels as observed by Bernard (18), Finsen (19), Hasselbalch and Jacobaus (20) probably stimulates diffusion between blood stream and tissue spaces. *2*, The hyperemia of the secretory organs caused by irradiation may hasten glomerular filtration. It is hoped that experiments now under way in this laboratory may help in the solution of these problems.

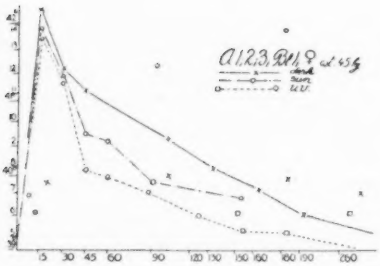

Fig. 1

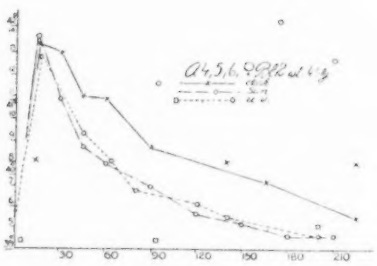

Fig. 2

Fig. 1. Experiment. *A 1*, July 17, 1923; *A 2*, July 21, 1923; *A 3*, August 8, 1923.
Fig. 2. Experiment. *A 4*, July 25, 1923; *A 5*, July 28, 1923; *A 6*, August 1, 1923.

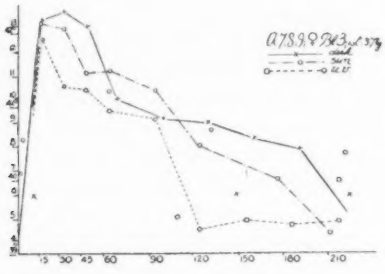

Fig. 3. Experiment. *A 7*, July 19, 1923; *A 8*, July 24, 1923; *A 9*, August 2, 1923.

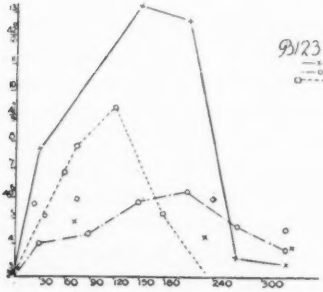

Fig. 4

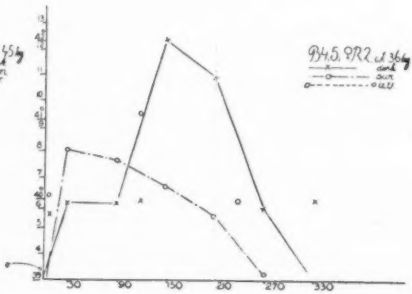

Fig. 5

Fig. 4. Experiment. *B 1*, August 7, 1923; *B 2*, August 10, 1923; *B 3*, August 14, 1923.
Fig. 5. Experiment. *B 4*, August 9, 1923; *B 5*, August 13, 1923.

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ACTION CURRENTS FROM THE STOMACH

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In connection with some behavior work on the relation of the stomach activity, tone and movements, to gross bodily activity and emotional conditions of fear, fright and gastric neuroses, I had occasion to make a few observations on the more strictly physiological aspects of the functions of the stomach. This digression into the field of physiology was made because of difficulties encountered in the behavior investigations. These difficulties seemed to be due very largely to our inadequate knowledge, despite the great amount of data collected by Cannon, Carlson and others on the simple physiology of the stomach. I have in mind here only that part of the stomach physiology which has to do with the action of the muscle walls, the changes in tone, the movements of the digesting and empty stomach, etc.

Part of the difficulty seemed also to be due to the method that was used for recording the changes in the stomach tone and movements—the balloon method, with which are recorded the changes in pressure in a balloon introduced into the stomach. This method registers the stomach movements and inhibition of movements fairly well, but it does not record satisfactorily the changes in tone, which is very important for the particular kind of behavior investigation that was being carried on.

The method used in the present work was that of leading off the action currents from the stomach walls during the various phases of activity and inactivity. This method has so far been used to the greatest advantage in the study of smooth muscle reactions by Orbeli and Bruecke (1) in their work on the action currents from the ureter of dogs. There has been a considerable amount of work by others, but the work of these two investigators was the first to show that well-defined negative and positive variations accompany the passage of each contraction wave down the ureter. There is an additional interest at the present time in the attempt to obtain a working knowledge regarding the action currents from smooth muscles, because of the recent hypotheses that have been set forth by a number of workers, chiefly Boeke and de Boer, on double innervation of striped muscle. The attempt has been made to explain the prolonged maintenance of postures of cataleptic patients without any apparent fatigue, on the basis of the double innervation of the striped muscle and the presence in these muscles of a tissue which has all the

characteristics of smooth muscle. It would be quite desirable, then, to be able to make a satisfactory study of this interesting problem by means of the action currents. So far, most all attempts to show the presence of a tonus mechanism characteristic of smooth muscle tissues in the striped muscle by means of the action current method have given negative results.

The following investigations were made on the exposed stomachs of twelve etherized dogs. A few experiments were made on cats and guinea pigs, which did not give very satisfactory results. In the dog an incision

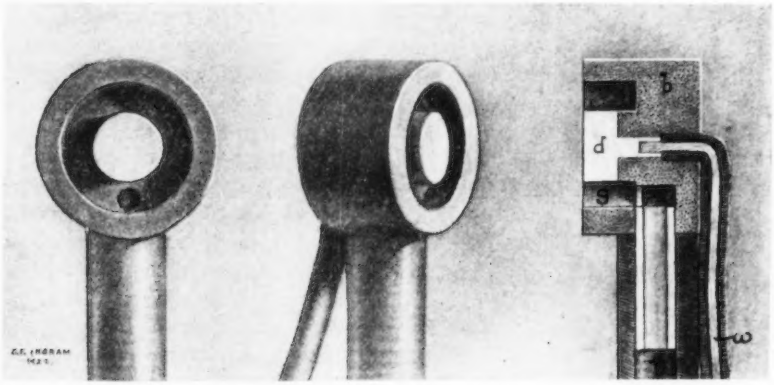

Fig. 1. Three views of suction disc electrodes, twice natural size. Dimensions; outside diameter, $\frac{1}{4}$ inch; depth, $\frac{1}{16}$ inch; diameter of inner zinc disc, $\frac{1}{8}$ inch; the flat surface of the zinc disc, *d*, is pressed firmly against the stomach wall, and then the air is sucked by means of a faucet suction pump from the groove, *g*, through the tube, *t*. In this way the electrode is attached, and the zinc disc is held in firm contact with the stomach wall. This disc is completely insulated from outside electrical disturbances by the hard rubber walls of the electrode and the rubber sheath of the leading-off wires. The rubber tubing containing the wire, *w*, is carefully fitted into the electrode and the place of junction is carefully sealed with shellac.

was made along the line of the ribs on the left side of the midline, and then across a short distance to the right side. Care was taken to make the incision as bloodless as possible, as it was found that the presence of blood on the stomach walls greatly interfered with the efficiency of the electrodes. The electrodes were attached immediately after the opening was made. Then the opening was covered with cotton soaked in a warm salt solution, and a hot-water bag was placed under the animal's back. All of the animals were starved at least a day, but more often two or three days, before the day of the experiment.

The electrodes used were especially designed for this purpose. They were made so that they could be easily attached to the stomach without

doing injury and also so that they would conform to the movements of the stomach without causing changes in contacts and resulting changes in electric currents. Three views of one of the electrodes used are shown in figure 1. This electrode is built on the plan of the suction disc cup used by Lashley (2) for collecting the secretion from the parotid gland. The electrode is placed against the stomach wall and then the air is sucked from the inner groove by means of an ordinary suction pump. Only a small suction pressure is needed to hold the electrode firmly in place. No injury is done to the walls of the stomach by the suction process. The inner part of the electrode is made of zinc. A thin and very flexible wire is connected to this zinc disc through the top of the electrode. The body of the electrode is made of insulating material, either Bakelite or hard rubber. The latter is more satisfactory, especially when the electrodes are made very small. It is very important to make the electrodes as small as possible because the walls of the stomach are often subjected to such violent changes in conformation during the passage of the contraction waves. The smaller the electrodes are, the better they ride the waves without causing changes in the currents due to changes in contact. The electrode shown in figure 1 is $\frac{1}{4}$ inch in diameter and is $\frac{3}{16}$ inch in depth. The central zinc disc is $\frac{1}{8}$ inch in diameter. In the first few experiments a very much larger electrode was used. It was $\frac{3}{4}$ inch in diameter and $\frac{3}{4}$ inch in depth. It gave fairly good results when the stomach was not very active, but tended to become dislodged during severe contractions. It had another disadvantage in that the groove between the zinc disc and the body of the electrode was large enough to permit the muscular tissue to be drawn up into it by the suction, thus frequently causing injury to the walls. In the small electrode where this groove is very narrow this defect is satisfactorily eliminated. The wire leading off from the electrode is completely insulated along its entire course by very soft and flexible rubber tubing. The place of contact with the electrode is carefully sealed with shellac. In this way the zinc disc is completely insulated from the surrounding organs. It was possible to work satisfactorily with the electrodes when they were attached to parts of the stomach lying deep down in the abdominal cavity and closely surrounded with other organs and tissues. The electrodes were non-polarizable. This was tested by observing the reaction of the string of the galvanometer when a constant current was passed through the electrodes attached to the stomach. The deflection of the string caused by the passage of this current was perfectly maintained, and there was no indication of a tendency to return to the original level.

The action currents were led off to a Hindle string galvanometer, the same model that is in use in this country for electrocardiography. The resistance of the string was 6700 ohms. Most of the action currents

were taken with a fairly tight string, usually a 1 cm. string—i.e., without the stomach in circuit, one millivolt causes a deflection of 1 cm. on the scale. With the stomach in circuit the deflection of the string varied considerably from time to time. I made no consistent effort to obtain a standard deflection with the stomach in circuit, as is done in ordinary electrocardiograph work, for the reason that I was interested in the form rather than in the amplitude of the responses. The records of the movements of the string were made photographically.

In the beginning of this work both electrodes were attached to the stomach walls. It was found, however, that equally good results could be obtained with only one electrode attached to the stomach wall. The other was attached to some part of the outside surface of the animal, usually on the hairless area of the skin over the groin. With the second electrode in this position there is little interference from the heart currents. The suction disc electrode is replaced by a sheet of zinc an inch or two in diameter attached to the skin by means of a paste made of kaolin mixed with saturated zinc sulphate. This kind of electrode is also non-polarizable. Because the second electrode is some distance off from the stomach and on a fairly inactive part of the body it serves in very much the same way as the lead taken from the "killed" part of a nerve in work on monophasic action currents from nerves. There are electrical changes only under the electrode attached to the stomach and none under the other electrode.

Results. It was found that every contraction wave passing along the stomach wall is accompanied by a series of action currents consisting of a number of different phases of varying duration and amplitude. These phases can best be studied when only one electrode is attached to the stomach, while the other is attached to the outside of the body over the groin. With this attachment of the electrodes the action currents most frequently consist of three phases; two quick phases followed by a comparatively slow and prolonged phase, but usually of the same amplitude as the quick phases. In figure 2a is shown a typical series of action currents obtained from the region just below the antrum and accompanying seven visibly observed contraction waves. The action currents that accompany just one contraction wave are shown in figure 2b. This record shows the two quick phases, one positive, the other negative and each of about $\frac{1}{2}$ second duration. Following these phases is a very much longer phase 5 to 6 seconds in duration. It will be noticed that there is an additional negative phase just following the negative quick phase. This phase is very irregularly present. It was not possible to discover any consistency whatever in its appearance.

The triphasic action currents such as I have just described are by far the most frequent, but in many records there is an additional phase which is quite important for the later understanding of the origin of these dif-

ferent phases. This phase may be seen in a series of action currents in figure 2c. This record shows the quick positive and negative phases

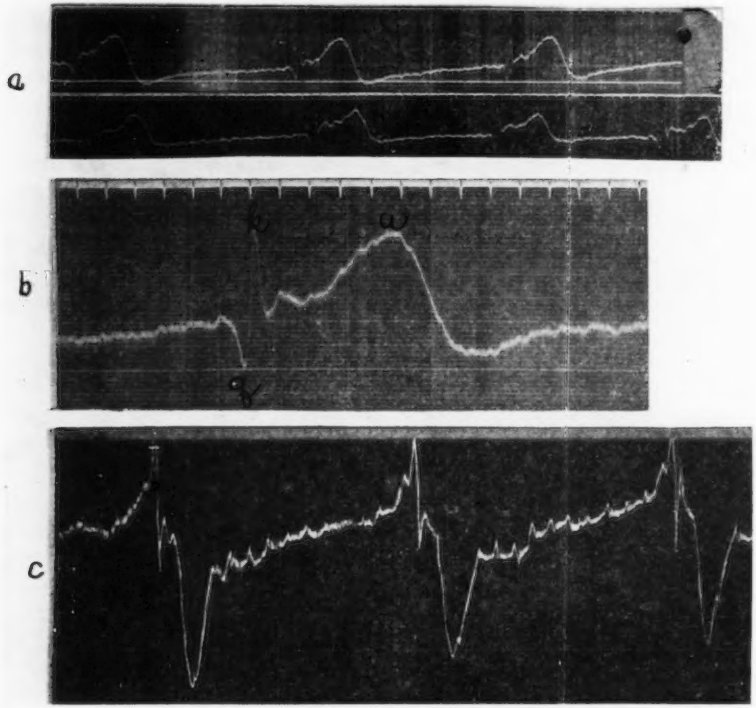

Fig. 2a. Typical record of action currents from the stomach with single electrode attached just below the antrum, the other electrode being attached to the skin over the groin. The top record shows action currents accompanying three contraction waves, the lower record shows the action currents accompanying four contraction waves. Time in seconds. Negative variations are to the top of the record, positive to the bottom.

Fig. 2b. Typical records of action currents accompanying single contraction wave. There are two quick phases, q and k, and a slow phase, w. In this record there is an additional quick phase just following the quick phase, k.

Fig. 2c. Action current records in which four distinct phases accompanying each contraction wave are shown. The two quick phases and the slow phase are present as in figure 2b, but there is an additional slow positive phase which precedes the slow negative phase. The two quick phases are superimposed on this slow phase. Time in seconds. Positive variations are to the top of the record, negative variations to the bottom.

followed by the slow negative phase, but it also shows a slow positive phase which precedes the slow negative phase. The quick phases are superimposed upon it and tend to make it stand out less clearly.

For the convenience of further discussion of these different phases I wish to designate them with the letters shown in figure 2b and in figure 3a.

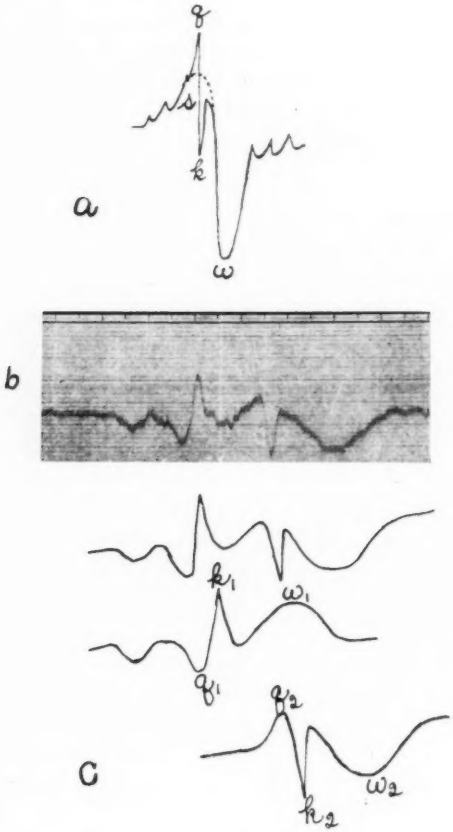

Fig. 3a. Tracing from one of the action curve records shown in figure 2c, with designation of the four phases.

Fig. 3b. Records showing action currents accompanying passage of single contraction wave when both electrodes are attached to the stomach and in the longitudinal line of the stomach.

Fig. 3c. Tracing of records shown in figure 3b, with the component parts contributed by the changes under each of the two electrodes drawn separately. The changes first reached by the contraction wave and designated as q_1, k_1, w_1. The changes under the second electrode are designated as q_2, k_2, w_2. Time in seconds. Negative variations are toward the top of the page, positive toward the bottom.

When both electrodes are attached to the stomach in the line of the passage of the contraction wave similar action currents are obtained, but of course there are two sets of quick-slow phases—one set when the contraction wave passes under the top electrode, the other set when the wave passes under the second electrode. The second set will be inverted with regard to the first. A typical record of this kind is shown in figure 3b. In this curve the slow phase *s* is absent. The individual components of this curve are shown in figure 3c. The quick-slow phases are present in both sets of currents. The distance between the electrodes was about $\frac{3}{4}$ inch. By measuring the time interval between any two homologous peaks it is possible to obtain the speed of the contraction wave, which usually was found to be $\frac{1}{4}$ to $\frac{1}{2}$ inch per second.

The amplitude as well as the form of the different phases depends on the position on the stomach from which the action currents are led off. Often only the slightest change of position of the electrode, $\frac{1}{4}$ inch or even less, is sufficient to alter the shape of the phases quite markedly. It is not possible now to make any definite statements as to how the phases vary with the different parts of the stomach, except for the fact that the amplitude of all the phases is greatest near the pylorus and smallest near the cardia. The quick phases are apt to be somewhat larger than the slow phases at the pyloric end of the stomach, but above the antrum they become smaller very much more rapidly than the slow phases. On the fundus the quick phases are completely absent while the slow phases may still be obtained. Attention may be called at this point to the fact that the longitudinal and circular layers of muscle fibers of the stomach are thickest between the antrum and pylorus and that they become very much thinned out on the fundus. The distribution of the longitudinal fibers on the fundus is limited very largely to the area near the greater and lesser curvatures. In a number of experiments it seemed that the quick phases were obtained for a greater distance above the antrum near the two curvatures, but these observations are not very certain.

The presence of the different phases varies with the condition of the stomach, according to whether it is in a contracted or relaxed state to begin with. In several cases it appeared that when the former condition prevails, the quick phases are present alone while the slow phases are absent. When the stomach is very much relaxed it appeared that only slow phases are obtained. In this connection it may be pointed out that the action currents from the stomach of the cat show only the single slow phase. A record from the cat's stomach is shown in figure 4a. Experiments were made both on etherized and decerebrate preparations. I am inclined to explain the absence of the quick phases as being due to a more or less chronic relaxation of the stomach of cats following etherization or decerebration. I have waited up to eight hours after the opera-

tion to make sure the effect of the shock was worn off, but the record remained the same. In this connection I should like to mention that in a decerebrate cat on which I took stomach contraction records at intervals for six days I did not find any kind of stomach activity except occasional small tonus changes. This may throw some light on the above findings.

Furthermore the action currents vary according to whether the stomach is fatigued or not. When the stomach is in good condition at the begin-

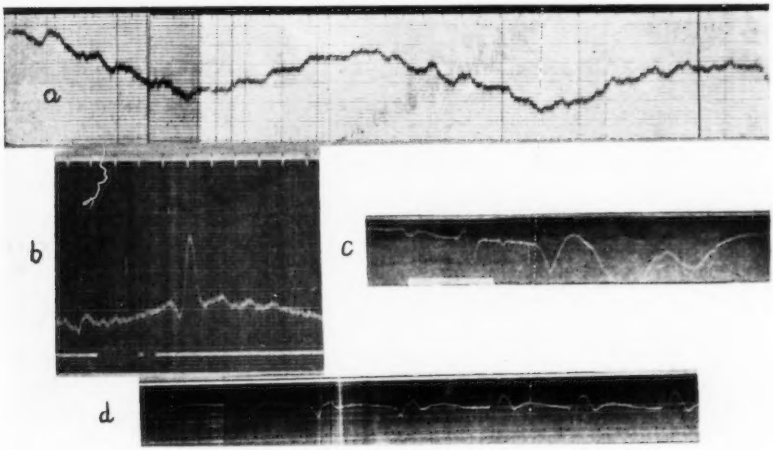

Fig. 4a. Record from the stomach of cat, showing only slow phases. Time in seconds.

Fig. 4b. Action currents following stimulation of peripheral end of cut vagus. The signal of the stimulation of the vagus is shown at the bottom of the record. Time in seconds. Records show that only quick phases of the action currents were present. They follow the beginning of the vagus stimulation after a limited period of 3 to 4 seconds.

Fig. 4c. Record obtained under same conditions as 4b, showing electrical changes that take place 10 to 12 seconds after beginning of vagus stimulation. In this record the two small dents in the curves at the beginning and at the end of the vagus stimulation are due to disturbances in respiratory movements caused also by vagus stimulation.

Fig. 4d. Record of action currents showing occasional extreme irregularity in shape of curves from one contraction wave to the next. Time in seconds.

ning of an experiment on a healthy dog the quick phases occur usually in a definite temporal relation to the slow phases, that is, they just precede the latter. When, however, the stomach is very much fatigued, as, for instance, after a whole day's experiment, this relationship breaks down and the quick phases may come almost at any time from the beginning to the end of the slow phases.

It appears from these findings that the quick and slow phases are due to separate processes which usually function together, but under abnormal conditions may become dissociated. This fact may be kept in mind in consideration of the analysis presented below.

More information regarding these different phases was obtained from some experiments in which the action currents were recorded following stimulation of the peripheral end of the cut vagus. It was found that stimulation of the vagus is followed after a latency period of 2.5 to 4 seconds by quick phases of action currents alone, either only the negative phase k, or q and k together, but the slow phase is absent in every case. It is not until some 10 to 20 seconds after the beginning of the stimulation that the regular action currents including the slow phases appear. At this time the currents are very large and somewhat distorted by the effect of the vagus stimulation. A typical record of this kind is shown in figures 4b and 4c.

There is another fact regarding the spontaneous action currents that must be pointed out. Very often the shape and form of the action currents are exactly the same from one wave to the next for many waves in succession. Sometimes, however, the oddest and most sudden transformation of the currents takes place from one wave to the next. A record of this kind is shown in figure 4d. Usually the successive currents show the same constancy that is shown in figure 2a. When both electrodes are attached transversely on the stomach so that they are both reached by the contraction wave at the same time, the form of the action currents is rather different from currents obtained with the electrodes in the line of the contraction wave. Sometimes with this attachment there are no currents at all as might be expected if the electrodes are exactly on the same transverse line.

It might be pointed out that these action currents that I have described above are quite different from the currents described by Alvarez (3), who used a slow responding galvanometer and failed entirely to obtain the quick phases q and k. The fact that I obtained these phases may be partly due to the intimate contact between muscle and electrode that was made possible by the kind of electrode that I used. Where Alvarez by means of two electrodes attached to the stomach recorded at most only two phases, I was able to record six, and sometimes eight phases.

Alvarez has made the interesting suggestion of the presence of a mechanism in the stomach that has at least some of the characteristics of the pacemaker of the heart. He arrived at this idea, as I understand it, chiefly from the results of his work on the changes in rate of the action currents in different parts of the stomach. He also regards the changes in amplitude of the action currents, especially of the positive and negative phases which he occasionally obtained, as an indication of the shifting of the

pacemaker. In the present work I did not look especially for changes in rate of the currents. I was struck, however, by the extreme regularity rather than the irregularity of the rate. I did observe, when the electrodes remained undisturbed in the same position, that there were oc-

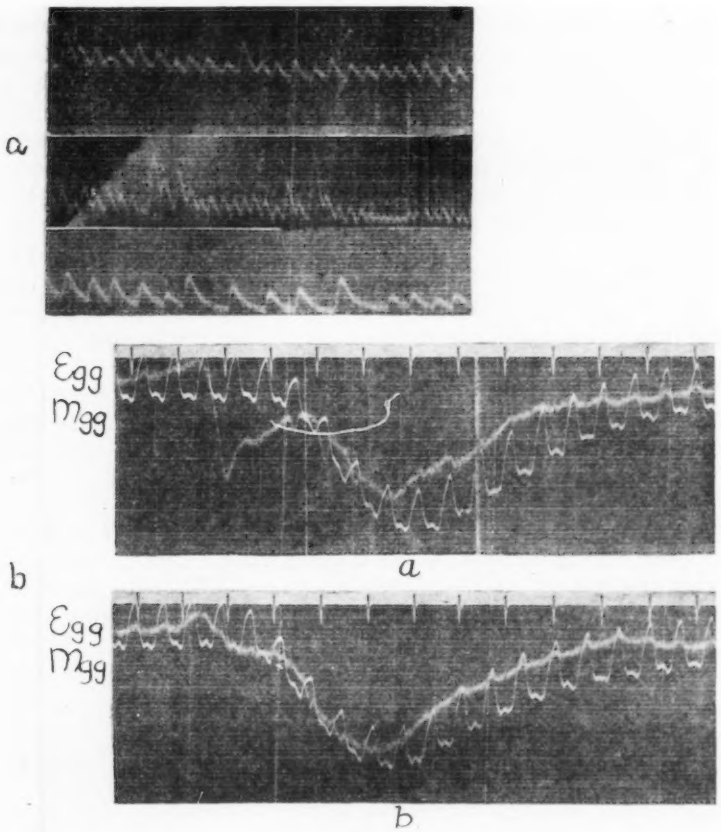

Fig. 5a. Records showing probable artefact action currents obtained from the dog with both electrodes attached to the stomach. These currents remained the same whether the animal was grounded or not. Time in seconds.

Fig. 5b. Stimultaneous mechanical and electrical record of passage of contraction wave. Time in seconds.

casionally very sudden, unexplained changes in the form and amplitude of the different phases from one current to the next, as is shown in figure 4d.

I wish to describe here some action currents, possibly artefacts, that were obtained while working on the stomach, which are completely different from

the action currents that were described above. These currents were obtained with exactly the same methods and general technique, the only exceptions being that a large difference in potential between the two electrodes was always present. Ordinarily this difference in potential amounted to only a few millivolts or often there was no difference at all. But always when the artefacts were obtained this potential difference amounted to several thousand millivolts. This difference was compensated in the usual manner. The other exceptions to the ordinary conditions of the experiment are that in all cases the dogs were unusually fat so that the abdominal cavity was flooded with omentum and also that considerable amount of blood had been spilt on the stomach in making the incision. There is a probability that there was blood under the electrode. Some typical records obtained in this way are shown in figure 5a. The time is indicated at the top of the record in seconds. The rate of these currents varies from 15 to 55 per second. It is very difficult to explain them on any other ground than disturbances from outside sources. I took care to ground the animals each time this kind of a record was obtained, but grounding did not in any way influence the currents. The room in which the galvanometer is kept is quite distant from the hospital motors. The marked change in their rate and amplitude from one moment to the next, makes an explanation difficult on a strictly mechanical, non-physiological basis.

Analysis. The first step in the analysis of the origin of the different phases of the action currents is to establish their relation to the passage of the contraction wave. For this purpose simultaneous mechanical and electrical records of the contraction wave were taken. The mechanical records were obtained by recording the shortening of the fibers between two points on opposite sides of the stomach equally and simultaneously affected by the passage of the contraction wave, that is, as nearly as possible on the same band of circular fibers. One of the points was made fast while the other was free to move. A hook with a thread attached was fastened to the movable point. The thread was connected to a small, very light aluminum lever adjusted in front of the slit of the galvanometer camera. The lever was counterbalanced just enough to ensure an easy and quick following of the contractions of the stomach muscles without putting any stress on these muscles. The electrode was adjusted just midway on the line between the two points.

Records obtained in this way show that the slow phase w of the action currents coincides in form and amplitude with the passage of the contraction wave. The quick phases precede the contraction wave and are in almost every case complete before the actual contraction begins. A record of this kind is shown in figure 5b. It will be seen that the two quick phases, q and k, occur before the beginning of the contraction and that

the slow wave, *w*, coincides fairly closely with the contraction. In figure 5b, a record is shown in which the coincidence between the slow phase and the contraction is almost perfect. The coincidence becomes the more perfect the more nearly it is possible to get the electrode and the two points from which the mechanical records are taken on the same line and on the same band of circular fibers. In this record the quick phases are absent.

The perfect correspondence between the mechanical and electrical records shown in figure 5b, would seem to point to the possibility that the slow phases are not action currents after all, but only electrical changes due to changes in contacts of the electrodes, in short, that they are artefacts. I feel reasonably certain that the electrodes that I used are not affected sufficiently by the passage of the contraction wave to bring about any changes in contact. I have tried to reassure myself on this point by pulling on the electrodes and then noting the electrical changes. It is only with very severe pulling that any changes appear and these are relatively small. There is the further fact that often when the contraction waves passing over the stomach walls are very slight, and when the electrodes remain perfectly motionless and undisturbed, the slow phases are obtained just the same as at other times. This finding made me think at first that action currents are obtained from the stomach in the absence of actual contraction of the muscles in the same way, according to the work of Mines, that they are obtained from the heart. With delicate levers I found, however, that a contraction accompanies every action current. Einthoven has shown recently that this is true also in the heart.

The mechanical records of the passage of the contraction wave shown in figure 5b have the limitation of showing only the contraction of the circular fibers and fail to show what is going on in the longitudinal fibers, which form, of course, a very important part of the stomach musculature. It is a common opinion that these two layers of fibers function synchronously and inseparably, but the evidence on which this opinion is based leaves considerable room for other possibilities.

Therefore, attempts were made to obtain records also of the contractions of the longitudinal fibers, with relation to the action currents. I wish to point out immediately that it is not a simple matter to obtain mechanical records of the contractions of the longitudinal fibers which are free on the one hand from the effects of the contractions of the circular fibers, and on the other hand from the effects of the respiratory movements. The interferences from the contractions of the circular fibers are quite difficult to eliminate, when records are taken in this way on the intact stomach in situ. There is an additional difficulty which is possibly even greater than the disturbance from the circular fibers. This is the matter of selecting the position of the points on the longitudinal fibers between

which the contractions are to be registered. It is entirely unknown at this time just what lengths of longitudinal fibers are involved at one time during the passage of a contraction wave. Curiously, this point has never been investigated. It is obvious that the length of the longitudinal fibers involved is not as long as the distance on the stomach that is affected by the contraction of the circular fibers, for if it were, the longitudinal and circular fibers would tend to work against each other, which seems rather improbable. This is shown in the sketch in figure 6b. This sketch shows a contraction wave *abc*. If the longitudinal fibers along the entire length of the contraction wave *abc* are in a state of contraction at the same time, then there would be a tendency for the distance *abc* to be shortened and straightened by the contraction. The contraction of the circular fibers tends to lengthen this distance. The longitudinal contractions would tend to eliminate the effect of the contractions of the circular fibers, and thus the longitudinal and circular fibers would be opposing each other in their action. Obviously a considerably shorter length of the longitudinal coat is affected by the contraction wave at one time, but just how short a length? And what is its position with relation to the contraction wave of the circular fibers? From the sketch it would seem probable that at the point *b* of the contraction wave the longitudinal fibers are in a condition of relaxation rather than contraction. In order to obtain an accurate record of the contractions of the longitudinal fibers it is clear then that the distance between the points in which the records are taken must include only the length that is involved at one time by the contraction wave. Our attempts to determine this distance by mechanical means were not successful, chiefly because the closer the two points are brought together the more delicately the registering devices must be adjusted, and consequently the greater are the interferences to this delicate adjustment from the contractions of the circular fibers.

The fact that the length of the longitudinal layer involved at one time in the contractions is probably very short compared with the length of the circular layer involved at one time, and the fact that a similar relationship holds true also for the durations of the quick and slow phases of the action currents, suggest a relationship between the longitudinal contraction and the quick phases of the action currents. This same relationship was suggested, however, almost immediately by the records shown in figure 5b by which the quick phases were left unaccounted for by the contractions of the circular fibers. The difficulties of the technique of recording contractions of the longitudinal fibers have greatly hindered the attempts to test out this suggestion. In a few experiments, however, it was possible to obtain rather definite information regarding this relationship.

In these experiments simultaneous tracings were taken of the action currents and the contraction of the longitudinal fibers, not during the passage of the contraction wave, but during the response of the whole

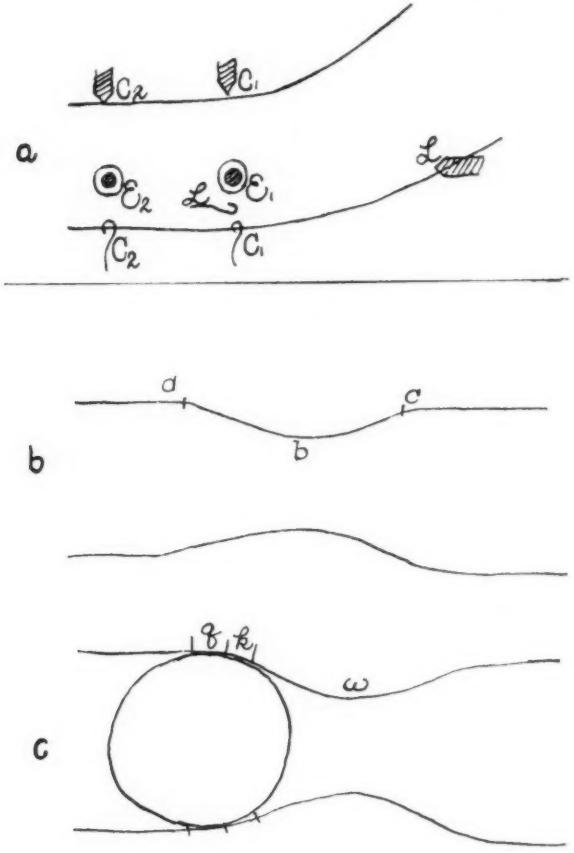

Fig. 6a. Diagram of attachment of electrodes and recording device used in vagus stimulation experiments. The electrodes are marked *E*. The places of attachment of the recording devices in the circular fibres are marked *C*, of the longitudinal fibers, *L*. The fixed points are indicated with pointed, shaded areas.

Fig. 6b.

Fig. 6c. Sketch showing approximate relationship of contractions of longitudinal and circular coat based on findings of present work.

stomach following stimultation of the peripheral end of the cut vagus. Along with these tracings contractions of two rings of circular fibers were

recorded. The places of the attachments of the electrodes and the points for registering the longitudinal and circular contractions are shown diagrammatically in figure 6a. The distance between the points in the longitudinal fibers was $1\frac{1}{2}$ inches. The placing of these two points with relation to the electrodes was rather unfortunate for the reason that whatever changes come down the stomach walls will affect the longitudinal registering devices first and make the time relation between the longitudinal contractions and the electrical records rather difficult to read. With this arrangement of electrodes and recording devices the record shown in figure 7 was obtained. In these records it will be seen that the stimulation of the vagus is followed by the diphasic action current shown in figure 4b. There are two diphasic, however, in this case, separated by a four-second interval, one associated with the changes that take place under the first electrode, and the other with the changes that take place under the second electrode. The slow phases are absent, as is usual, following vagus stimulation. It will also be seen that these quick phases

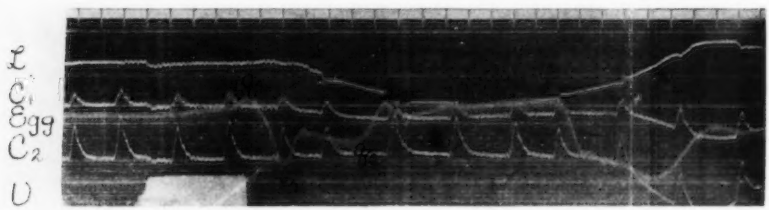

Fig. 7. Vagus stimulation experiments. Records showing relation of action currents to contraction of circular and longitudinal coats.

occur in the absence of any changes in the circular fibers. There is, however, a well-marked contraction of the longitudinal fibers. The time relation between the quick phases and the contraction of the longitudinal fibers is not clear because of the unfortunate position of the recording devices. Despite this shortcoming the records seem to show quite definitely that the quick phases are associated with the contraction of the longitudinal fibers.

The final proof of this relationship will have to wait until simultaneous records are obtained of the action currents and of the contractions of such short lengths of longitudinal fibers as are involved at one time by the passage of the contraction wave. Assuming the truth of the relationship the actual length of the longitudinal fibers affected at one time may be easily determined from the electrical records. Given the duration of the quick phase k equal to $\frac{1}{2}$ second, and the speed of the contraction wave equal to $\frac{1}{4}$ to $\frac{1}{2}$ inch per second;

Then: Distance = Time $\times$ Velocity = $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ inch.

According to this equation $\frac{1}{4}$ inch or less of the longitudinal fibers will be affected at one time. This equation is calculated for the negative phase *k* which is produced during the contraction of the fibers. The preceding positive phase *q* which is produced by relaxation of the muscle is of a similar duration, so that the relaxation wave that precedes the contraction will also involve about $\frac{1}{4}$ inch or less of the longitudinal fibers at one time. According to this theory the action of the longitudinal fibers during the passage of a contraction wave may be pictured then in the following way shown in figure 6c. In this diagram the relaxation of the contraction phases of the longitudinal fibers just precedes the beginning of the contraction of the circular fibers. The relaxation phase probably just precedes the point of purchase of the muscles on the bolus of food, while the contraction just follows it.

The different phases of the action currents shown in figure 5 can thus be interpreted as follows. The quick positive phase can be interpreted as a relaxation phase preceding the contraction of the longitudinal fibers and the slow phase *s* as a relaxation preceding the contraction of the circular fibers. The fact was pointed out that the slow positive phase is often very small or is not present at all. The record shown in figure 5 in the work of Bayliss and Starling (4) on the movements of the intestines shows that the relaxation wave of the circular fibers also is less prominent in the intestines than the relaxation of the longitudinal fibers. This record was obtained by means of specially devised enterographs which register the contractions of the two coats of fibers simultaneously. A bolus was introduced into the intestine a short distance above the place of attachment of the enterographs, and the changes in the two coats were registered during its passage down the gut. It shows that the preceding inhibition of the circular coat is very small, also that this relaxation wave begins about three seconds before the onset of the contraction and increases very gradually, while the relaxation of the longitudinal fibers begins less than a second before the contraction, increases very rapidly, and reaches a large amplitude. There is a striking similarity between the action current records from the stomach shown in figure 5 and the mechanical records from the intestines shown in the record referred to above from the work of Bayliss and Starling.

Up to the present time there has been little or no evidence as regards the separate function of the longitudinal and circular coats of fibers of any of the hollow viscera. Most workers who have studied the action currents from these organs have tried at one time or another to interpret their findings in terms of the separate contractions of the longitudinal and circular layers, but always without success. Orbeli and Bruecke in their work on the action currents from the ureter of dogs attempted to

explain one of their quick phases in the terms of the contractions of the longitudinal layer. They finally decided against this interpretation and concluded that the two layers are equally and simultaneously involved in the production of the different phases of the action current. None of the evidence that has been obtained so far against the separate function of the two layers is based on experimental data. The data that I have mustered are by no means conclusive, but seem to point very strongly in the direction of the interpretation of the separate functioning.

The possibility must be considered here also that the quick phases are produced by a mechanism which contains muscle elements having some of the characteristics of striped muscle, chiefly a very much quicker form of response than is usually found in smooth muscle. The duration of the quick phases shown in the records is considerably shorter than any of the responses of the smooth muscle of the stomach about which we have any information at the present time. It would be very important, of course, to know just what the relation is between the size of the electrodes and the duration of the phases.

The difference in the speed of the two types of action currents may be also interpreted on the basis of the presence of a conductile and a contractile process, similar to that found in the heart. According to this interpretation the quick phases would be associated with the conductile process, that is, a nervous or semi-nervous mechanism, while the slow phases would be associated with the contraction of the muscles.

I should like to call attention to the fact that the findings in the experiments described above, in which the action currents were recorded following stimulation of the vagus nerve, suggest the presence of a hitherto overlooked response of the stomach. In these experiments evidence was found, it seems to me, for the presence in the stomach of a mechanism which controls the size and position of the entire stomach and which is separated in part at least from the mechanism that has to do with the passage of the contraction wave. It will be recalled that vagus stimulation was followed by the appearance of the quick phases alone without the slow phases. These quick phases appeared a very short interval after the stimulation, 2 to 3 seconds. That this reaction was probably not associated with the mechanism which has to do with the passage of the contraction wave seemed to be indicated by the fact that the regular contraction wave did not appear until some 15 seconds after the stimulation. I took mechanical records of the changes in size of the whole stomach following vagus stimulation, either by registering the shortening of longer lengths of the stomach (4 to 5 inches) by means of levers attached to the outside, or by the balloon method. I found that there is always a latency period of 2 to 4 seconds before the beginning of the first sign of change in size of the stomach. The coincidence in the time relations of these two

phenomena, electrical and mechanical, would seem to indicate a more intimate association between them.

According to the findings of the present work this mechanism would be contained in the longitudinal fibers, because of the fact that they connect one end of the stomach with the other, as having to do more with the shape and position than with the work of pushing the food along toward the pylorus. Certainly by far the greatest part of the work of digestion, if not all of it, is done by the circular fibers. The electrical records show that the longitudinal fibers do take part in the passage of the contraction waves, but they show that at times they become dissociated from the others. It must also be considered here that the quick phases obtained following vagus stimulation may be due to some special mechanism in the stomach walls, apart from the longitudinal fibers, which has to do exclusively with reactions that deal with changes in shape and position. At present there is no evidence at hand for the presence of such a mechanism.

The presence of a reaction involving the whole stomach should help to throw some light on the problem for the solution of which this present work was undertaken; that is, on the mechanism of the responses of the stomach in situations of emotional excitement as, for instance, in fear or fright. It is a fact of common experience that the responses of the viscera, especially the alimentary tract, play a very important rôle in reactions of this kind. There are usually wide discrepancies in the descriptions of different individuals of just what happens in the viscera at these times. There is a fairly general agreement, however, that the reaction follows the stimulating situation by only a very short interval. The time relations of the quick phases following vagus stimulation suggest a connection between the mechanism underlying these phases and the mechanism involved in the emotional reactions. I am well aware that in emotional excitement there are other probably more important muscular changes in the stomach that have their origin in stimulation from the splanchnics. I was unable to stimulate these nerves effectively so I have no records of action currents following splanchnic stimulation.

In this connection it is important to keep in mind the different functions performed by the stomach musculature. There is first the function of digestion which is carried on by the passage of contraction waves along the stomach wall. This activity is not connected with any sensation. Digestion goes on without in any way interfering with the behavior of the organism except where there is obstruction or something of that sort which involves other parts of the stomach than those involved in the simple digestion process. A second function of the stomach is to play a part in notifying the organism of the need for more food, or of actually stimulating the organism to go out and get more food. This activity is

carried on by the so-called hunger contractions which appear only when the stomach is empty. It appears that there are afferent end organs in the muscles or in the connective tissue involved in the hunger contractions and that these organs are stimulated during the contractions so that they send impulses to the rest of the organism. In the muscles involved in the digestion contractions there seem to be no such organs. The third function of the stomach is to contribute to the signalling or stimulating of the organism in situations of emotional excitement. On the basis of the present hypothesis the end organs from which this stimulation comes would lie in the muscles involved in the total responses of the stomach, or they may lie in the connective tissue affected by this special form of distortion of the muscle wall of the stomach. A reaction of this kind has been described by Barclay (5) in some radioscopic observations that he made on the responses of the stomach of nervous patients to emotional stimulation. He found that a sudden, unexpected slamming of a door was followed by a sudden drop of the pyloric end of the stomach. This drop amounted to several inches. Barclay explained these results on the basis of a sudden relaxation of the oblique fibers. In his work he did not exclude the possibility of other muscle fibers being involved in this reaction.

I have not taken the presence of the oblique layers of fibers into consideration in the discussion of the origin of the different phases of the action currents, chiefly for the reason that at this early stage of the investigation I feel it would too greatly complicate and confuse the results. This layer is farthest away from the electrodes and is not so prominent as the other two layers, so that this negligence may not be serious. That some of the phases and the irregularities of the action currents will later be shown to be due to the contraction of this layer seems quite probable.

With a definite understanding of the origin of these different phases of the action currents it is hoped that a better grasp of the normal and abnormal functioning of the stomach may be obtained. Very much the same methods as those applied to the study of the action of the heart in electrocardiography may be applied also to the study of the action of the stomach, especially the effect of drugs on the different phases. This study will be necessarily limited to the material that can be obtained from the direct attachment of the electrodes to the walls of the stomach. So far no adequate method has been developed of obtaining the electrogastrogram from the outside surface of the body, as is done in the electrocardiogram.

This work was started in the fall of 1920 and has been carried on intermittently since then. I wish to acknowledge my indebtedness to Mr. David Brunswick and to Dr. Ging Wang, who assisted me in the experi-

ments from the very beginning. I am indebted to Mr. Brunswick also for many helpful suggestions made regarding the interpretation of the action currents. I also wish to make use of this opportunity, the first that has presented itself, to express my thanks to Dr. E. P. Carter of the heart station of the Johns Hopkins Hospital for his kindness in teaching me the use of the string galvanometer.

SUMMARY

1. It was shown that an electrogastrogram consisting of 3 to 4 definite phases is obtained from each contraction wave of the stomach in the same way that the different phases of the electrocardiogram are obtained from the beats of the heart.

2. There are two quick phases, one positive, q, and the other negative, k, having a duration of $\frac{1}{2}$ to 1 second each, and two slow phases, one positive and the other negative, having a duration of 5 to 15 seconds each. These records are obtained when only one electrode is attached to the stomach walls, while the other is attached to a neutral part of the body, the skin over the groin, and serves as a monophasic dead electrode. When both electrodes are attached to the stomach in line of the passage of the contraction wave 6 to 8 phases are obtained, the additional phases being produced when the wave reaches the second electrode. These phases are of course inverted with relation to the changes that take place under the first electrode.

3. This work was done on the exposed stomach of etherized dogs. Specially devised electrodes were used for this purpose and the action currents were led off to a string galvanometer.

4. The factors which govern the shape and amplitude of the action currents were discussed. The most important of these factors are the position of the electrodes on the stomach walls, the condition of the stomach, whether in good condition or fatigued, etc.

5. Possible artefact action currents at the rate of 15 to 50 per second were described.

6. By means of simultaneous mechanical and electrical records it was possible to show that the slow phase w of the action currents coincides both in form and amplitude very closely with the contraction of the circular coat of fibers.

7. These records had the limitation that they showed only the contraction of the circular fibers and did not show what was going on in the longitudinal fibers. Attempts were made to obtain records also of the contractions of the longitudinal fibers, but most of these attempts with a few exceptions were unsuccessful because of difficulties of technique. In some experiments in which simultaneous electrical and mechanical records of both the circular and longitudinal coats were taken following

the stimulation of the peripheral end of the cut vagus fairly definite information regarding the relation between the quick phases and the contractions of the longitudinal fibers was obtained. It was found that each stimulation of the vagus is followed after a latency period of 2 to 4 seconds by only the quick phases, the slow phases being completely absent. It was found further that these quick phases are accompanied by marked contraction of the longitudinal coat of fibers in the absence of any change in the circular coat.

8. Evidence from this experiment and from comparison of the general shape and form of the action current records with the records obtained by Bayliss and Starling in their work on the movements of the intestine and formulated in the "law of the intestine" seemed to show that the quick phases of the action currents are connected with the contractions of the longitudinal fibers. The quick positive phase preceding the quick negative would be regarded then as accompanying a relaxation preceding the contraction of the longitudinal fibers, while the positive phase preceding the slow negative phase would be regarded as accompanying a wave of relaxation preceding the contraction of the circular fibers.

9. It was pointed out that definite proof of this relation between the quick phases and the longitudinal fibers would have to wait until more satisfactory records of the contractions of the longitudinal fibers are obtained. There are a number of factors which make it very much more difficult to obtain records of the contractions of this coat than of the circular coat. The most important of these difficulties is due to the fact that it is not known at present just what length of the longitudinal coat is involved at one time in the passage of the contraction wave. It can easily be seen that it is very important to adjust the recording device to this distance. It is obvious that it does not extend over the entire area involved by the contraction of the circular fibers, otherwise the two coats would oppose each other in their action. The distance that is actually involved at one time is probably quite short. Attempts to determine this distance by mechanical means were unsuccessful because of interferences from the contractions of the circular fibers. If it is true that the quick phases of the action currents are produced by the contraction of the longitudinal coat in the same way that the slow phases are produced by the circular coat, then the length of the longitudinal coat that contracts at one time can easily be determined. It was shown that this distance in the dog is about $\frac{1}{4}$ inch.

10. It was pointed out that the findings of the experiments on vagus stimulation argued also for a whole organ response of the stomach based on the contraction of the longitudinal coat of fibers which connect one end of the stomach with the other. The significance of the presence of such a mechanism for the study of the reactions of the stomach in conditions of emotional excitement was discussed.

11. It was also pointed out that by means of experimental study of the different phases of the action currents (effects of drugs, etc.) more information regarding the normal and abnormal functioning of the stomach may be obtained in the same way that the study of the electrocardiogram has helped to throw light on the action of the heart. At present there is no satisfactory way of obtaining these action currents from an intact animal.

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ATTEMPTS TO MAINTAIN THE LIFE OF TOTALLY PANCREATECTOMIZED DOGS INDEFINITELY BY INSULIN

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That insulin may control the diabetic symptoms and improve the nutrition of most diabetic patients, at least for a time, seems well established. These patients are partial diabetics. That is, on the assumption that all cases of clinical diabetes represent varying degrees of hypofunction of the pancreas, these have still some trace of functional islet tissue. It is otherwise with the totally pancreatectomized animal. If insulin administration can maintain such animals in good or even fair condition indefinitely, this would be conclusive evidence that insulin is or contains the essential and normal pancreas hormone. The investigation of this phase seems particularly important, both theoretically and practically. In the case of the best known and most extensively tried hormone, that of the thyroid, it is generally admitted that while thyroid or thyroxin administration *improves* the condition of cretinism and myxedema, it has not yet been shown that such hormone therapy can produce and maintain a physiological state equal to the normal in the man or animal having no thyroid tissue.

Banting and Best have reported keeping one pancreatectomized dog alive and in good condition for 70 days by insulin. In this dog a small portion of pancreas was found at autopsy, but it was not determined whether this had been left accidentally at the operation, or had regenerated from the pancreatic duct in the duodenal wall, as may occur according to the observations of Bensley on rabbits.

According to Doctor Carlson, completely pancreatectomized dogs survive for 15 to 50 days without insulin, depending on initial condition, on age, on diet, and on care of animal. Other investigators place the limit of survival of such animals at 12 to 15 days. It would seem that the length of life of depancreatized dogs under insulin, and the completeness of control of all the diabetic symptoms under such management should depend on whether insulin represents the entire pancreas hormone complex, and whether the insulin itself or the other organic substances in the insulin mixture may induce chronic injuries on prolonged administration. The present experiments were undertaken at Doctor Carlson's

suggestion with the hope of securing information on these two questions. We did not anticipate the complicating factor of regeneration of pancreas tissue from the pancreatic duct stump in the wall of the duodenum, rendered possible in dogs on prolonged insulin management.

Methods. Three dogs were totally depancreatized and insulin administered subcutaneously twice a day. Quantitative estimations of urine sugar were made daily by Benedict's method and the amount of insulin administered was based on the urine findings. We aimed to keep the daily excretion of sugar somewhere between 1 and 5 grams in order to prevent hypoglycemic reactions. All injections were made within an area about 3 inches in diameter behind the scapula. The site of injection was previously washed with alcohol. The needle was simply dipped in alcohol.

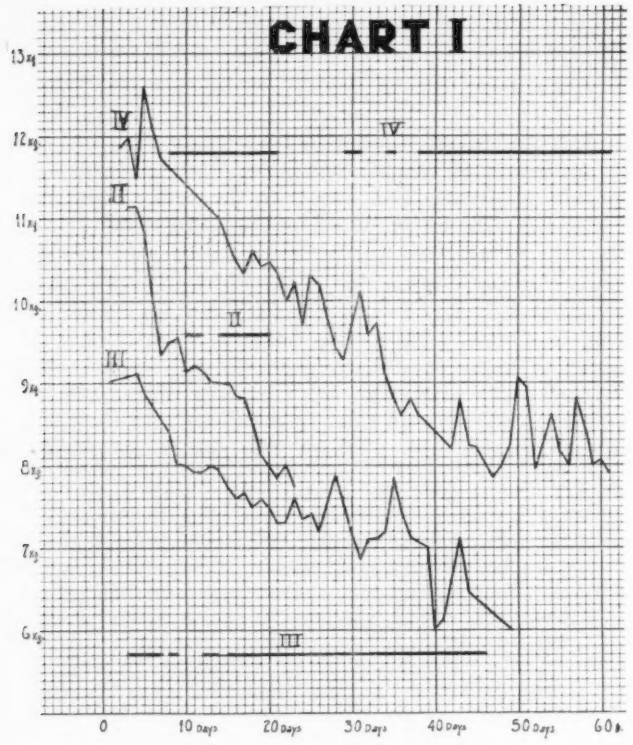
Three other totally depancreatized dogs had been used for studying the administration of insulin per vaginum and by Thiry fistula for several weeks, during which time their urine was never sugar-free for a period of more than 6 or 7 hours at a time. These dogs were then given insulin subcutaneously similar to the previous two cases with the idea of prolonging their lives.

At the end of the experiments, the six dogs were carefully autopsied by Doctor Carlson. When marked deviations from the typical diabetic condition occurred in any of the tissues they were submitted to microscopic examination.

Protocol of dog 1. Male, weight 13 kgm. Pancreas removed January 16. Subcutaneous administration of insulin begun January 23. The sugar in the urine was not absolutely controlled until February 9, when very potent insulin was made in our laboratory. The dog appeared much improved and made a slight gain in weight. Dog died by accident February 15. Autopsy findings—No trace of pancreas was found. All tissues were typical of a diabetic dog.

Protocol of dog 2. Male, weight 11.1 kgm. January 31, made a Thiry fistula. February 10, Doctor Carlson removed the pancreas. February 15, dog appeared in excellent condition. February 16, insulin administered by Thiry fistula. This method of administering insulin was continued until February 20. Then the urine was kept practically sugar-free by the subcutaneous method. Dog gradually became weaker with an increasing amount of blood in the stools. Dog died March 6. Blood sugar was 0.013. No insulin was administered for several days prior to death as the urine remained sugar-free. The corpuscles made up about one-tenth of the blood volume.

Autopsy findings—No trace of fat anywhere. Intestines,—cyanotic, filled with blood extending from the pylorus to the anus. Appendix,—markedly distended with gas. Thiry fistula, length 14 inches. Contained light brown cheesy material. Kidneys, right kidney appeared normal. Left kidney diseased. Nodular condition in cortical area. Heart, good. Lungs, in excellent condition, except for a few very small nodules. Liver, dark and fatty appearance when cut. Spleen, 2.5 inches long, $\frac{1}{2}$ inch wide, about $\frac{1}{8}$ inch thick. Pancreas, no trace of pancreas tissue. Death apparently due to asthenia of the gut. Food could no longer be absorbed.



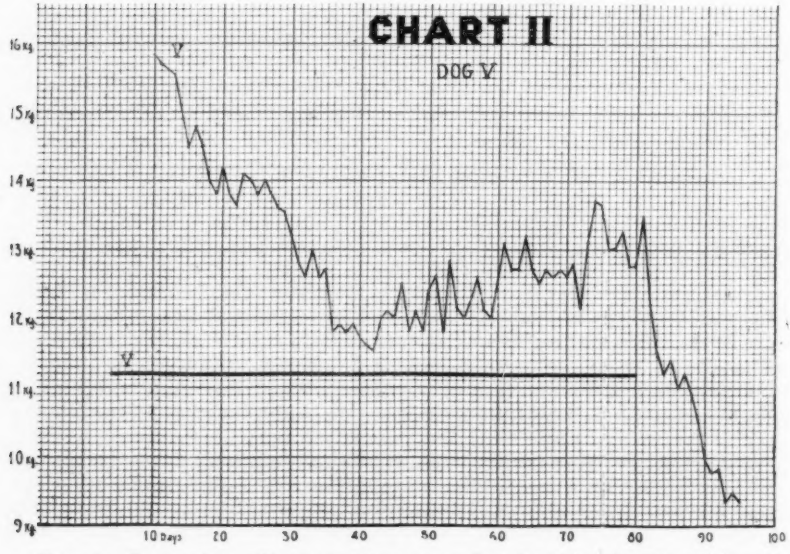


Chart 1. Dogs 2, 3, 4. Weight in kilograms recorded at left. Number of days at bottom. The graphic curves *2*, *3* and *4* represent the weights of three dogs. The heavy horizontal lines *2*, *3* and *4* represent the days on which urine of the corresponding dogs were kept practically sugar-free with insulin.

Chart 2. Dog 5. Weight in kilograms recorded at left. Number of days at bottom. Upper line represents weight curve of animal. Heavy black horizontal line corresponds to days on which animal's urine was kept practically sugar-free.

Protocol of dog 3. Female, weight 9.5 kgm., quite thin, but in apparent good health. February 28, enlarged vagina to facilitate the introduction of insulin into the uterus. March 6, dog in excellent condition. On March 7 the pancreas was removed. March 10, insulin administered subcutaneously. The urine was kept practically sugar-free until March 14. On this date vaginal administration of insulin was begun and the effects determined by the examination of the blood at intervals of an hour until the blood sugar returned to the original diabetic level. This procedure was followed from March 14 to March 22 with the exception of three days when insulin was administered subcutaneously. On March 22 the animal weighed 7.78 kgm. The dog was considerably emaciated and weak. Then insulin was given subcutaneously and the urine sugar was kept at about 1 gram per day. A severe diarrhea was observed on April 4. The mixing of bone ash with meat relieved the condition in two days. April 16, gave dog 15 grams dextrose with the meat and the insulin increased accordingly. The improved condition of the dog warranted the continuance of this treatment. April 25, 11 a.m., gave dog 200 cc. of milk and 11 grams of sugar by stomach tube. Sufficient insulin was given to burn the sugar. At 9 p.m. the animal appeared very weak. Death followed shortly afterward with no signs of coma. The legs moved constantly as in walking, while lying on the side. Respiration stopped suddenly and the heart a few minutes later. The dog seemed conscious to the end.

Autopsy: No trace of pancreas present. There were a few adhesions about the duodenum. Emaciation was very marked. All the organs appeared to be in an excellent condition.

Protocol of dog 4. Female about one year old, weight 12.5 kgm. March 28, made Thiry fistula. Dog recovered excellently. April 14, the pancreas was extirpated. April 18, wound almost healed and dog very playful. Insulin was administered by Thiry fistula and the blood sugar changes followed until April 22. Subcutaneous administration of insulin was then begun and continued until May 5. The dog was in fair condition, so insulin was again given by Thiry fistula until May 11, with the exception of one day. After May 11, the dog received insulin subcutaneously with the exception of four days on which the Thiry fistula method was again tried. The dog gradually became weaker and died on June 13 with no signs of coma.

Autopsy: Pancreas tissue about the size of a large headed pin present. Dog very emaciated. Spleen, extraordinarily small, 2.5 inches long, $\frac{1}{4}$ inch wide. All other organs appeared normal.

Protocol of dog 5. Male, very muscular and in good condition, weight 18.3 kgm. May 17, pancreas removed. Dog recovered very rapidly. On May 21 the wound practically healed, so it was not rebandaged. An hour later when I returned to the laboratory the dog had removed all the abdominal wall stitches except those holding the peritoneum together. The wound was cleansed with boric acid and dressed each day. Insulin was given subcutaneously. This afforded an excellent opportunity to test the influence of insulin on wound-healing. May 26, the wound had healed by second intention without any signs of infection. Sufficient insulin was administered twice a day subcutaneously to keep the urine practically sugar-free. June 11, insulin prepared by a different method was substituted. Four injections were made. Two days after each injection of this material a so-called sterile abscess appeared. As soon as the first abscess was noticed, insulin was substituted, from which the toxic fraction had been removed. No more abscesses appeared and the ones already formed disappeared in a few days. June 26, 9:30 a.m., injected 1.5 cc. of insulin prepared from calf pancreas which had not been standardized. At 11 a.m. hypoglycemic convulsions were observed and controlled by 20 grams of dextrose by stomach tube, recovery in 15 minutes. Convulsions reappeared at 1:00 p.m. Gave 15 grams dextrose by stomach tube with recovery, but convulsions returned an hour

Chart 3. Dog 6. Weight in kilograms recorded at left. Number of days at bottom. Upper line represents weight curve of animal. Heavy black line corresponds to days on which animal's urine was kept practically sugar-free.

later. Then 40 grams of dextrose were given by stomach tube. Dog recovered in about 10 minutes and ate a large quantity of meat. June 27, no trace of sugar was detected in the urine until the sample voided at 5:30 p.m. That is, the urine remained sugar-free for 34 hours following this one injection of insulin and during this time the dog received 75 grams of sugar by mouth and had eaten several pounds of lean meat. One-half cubic centimeter of this lot of insulin, given twice a day, kept the urine practically sugar-free. June 29, 43 days after pancreatectomy, the dog began to gain weight consistently. With the increase in weight the dog became more playful and appeared more like a normal animal. August 6, insulin treatment was discontinued. The dog was in such excellent condition that it seemed probable that he could be kept indefinitely as long as insulin was administered. August 21, at 10 p.m., there were signs of approaching coma. August 22, 8:30 p.m. died in coma.

Autopsy: Duct of Santorini greatly hypertrophied. The linen thread used to ligate the duct was found in the lumen proximal to a small piece of pancreas tissue, which had apparently regenerated from the pancreatic duct. This piece of fresh tissue weighed 61 mgm.

Stomach mucosa covered with small ulcers, not due to post-mortem changes. Intestines, no ulcers. No striking variations from a typical diabetic condition were noticed in the other organs.

Protocol of dog 6. Male, muscular and in excellent condition, weight 16 kgm. January 15, removed pancreas. On January 20 animal appeared in good condition, wound almost healed, blood sugar 0.500 per cent. February 7, subcutaneous administration of insulin was instituted. This procedure was followed until February 16. During this time there was a slight gain in weight. February 17, treatment discontinued. No insulin was given until February 27. During this period of ten days—33rd to 43rd day, on chart 3—there was a loss in weight of 1.6 kgm. Insulin was administered continuously by the subcutaneous method, from February 27 to April 5. In this 36-day period of treatment—43rd to 79th day on the chart—there was a 2 kgm. gain in weight. Insulin treatment was stopped for 22 days, during which time there was a loss in weight of 2.2 kgm. The dog became very weak and depressed. Beginning April 27 insulin was administered continuously for 46 days—102nd to 148th day on the chart. There was a gain of 1.2 kgm. The dog appeared to be in good condition but did not gain weight rapidly. Beginning June 12 the insulin treatment was again stopped for 24 days with a resulting loss in weight of 2.5 kgm. On July 7 the dog weighed 1.3 kgm. less than at any time previous. Insulin injections were again instituted. The urine was kept practically sugar-free for 28 days, in which time the dog gained 2.7 kgm. That is, the gain during these 21 days was greater than the loss during the preceding 24 days. As it seemed probable that the dog could be kept indefinitely, with insulin treatment, we discontinued the injections to determine how long the dog could survive. During the 43 days following the last administration of insulin, the dog lost 5 kgm., became extremely emaciated and weak. September 15, the dog although still able to walk, and eating freely, was killed with ether and a careful autopsy made.

Autopsy: extreme emaciation. Pancreas, a piece of pancreas tissue covered entirely by the serosa of the upper part of the duodenum. A small duct led from it into the lumen of the duodenum. This pancreas had regenerated from the duct. The pancreas was triangular in shape with a length of 2 cm., width 1 cm., and thickness 0.5 cm. The large duct had been ligatured with linen thread when the dog was depancreatized. At autopsy there were no signs of the duct having been ligated. Upon examination the ligature was found in the lumen of the duct where it opens into the duodenum. The pancreas was distal to point of the ligation of the duct at the pancreatectomy operation. The regenerated pancreas weighed 515 mgm.

Liver, very yellow and moderately enlarged. Spleen, very small. Lymphatics, great engorgement of abdominal lymphatics. Cisterna chyli at point of bursting and lymph was bile stained. Digestive tract, appeared normal both internally and externally. No adhesions about duodenum. Adrenals, appeared normal, weight (both) was 1.62 grams. Kidneys, normal. Testes—atrophied to about one-third their normal size. Weight (both) 3.5 grams. Heart, valves normal. Yellow granules around base of valves and around opening of the coronary arteries. Pulmonary artery, normal. Aorta, covered externally by yellow (bile-stained) protruding calcium deposits. The deposits extended through the arterial walls. Granules do not exist below the diaphragm. All large arteries were involved above the arch up to the division of the common carotid. The deposits were noticed on the inside of the corresponding arteries. Thyroid, small, very hard, considerably atrophied. Weight (both) was 1.195 grams.

Discussion. From the above protocols we see that dog 1 was killed by accident on the 30th day after pancreatectomy. At this time he was gaining weight and his condition was improving. Dog 2 died on the 23rd day after pancreatectomy from asthenia of the gut. Dog 3 was thin at the time of pancreatectomy. Insulin was administered for 14 days per vaginum and the blood sugar changes determined. At the end of this period the dog had lost 1.7 kgm. On subcutaneous administration of insulin the loss in weight was less rapid, but there was a continuous loss in weight to the end. Dog 4 was used to study the effects produced on diabetes when insulin was administered by Thiry fistula. Samples of blood were drawn every hour during the course of each experiment, when insulin was given by the intestinal fistula. Insulin was administered by the intestinal route for several successive days and then during a period of three to four days was given subcutaneously. This was done to get a comparison of the effects produced by insulin when given by the different routes, and to prevent excessive strain on the animal by the withdrawal of too much blood. This procedure was followed for 38 days, at the end of which time the dog was very emaciated and it was thought best to start administration of insulin by the subcutaneous route. This dog, like dog 3, failed to gain. The lives of dogs 3 and 4 were probably slightly prolonged by the insulin, but they died in spite of the treatment, with no acetone bodies or sugar in the urine.

In the case of dogs 5 and 6, it seemed probable that we could have kept them alive indefinitely provided that the insulin injections had been continued. Dog 5 lost weight gradually in spite of the urine having been kept practically sugar-free continuously for the first 43 days following pancreatectomy. This loss in weight, however, was not as rapid as when no insulin was given. On the 44th day he began to gain weight consistently on the same diet, and with the increase in weight his general well-being was markedly improved. Eighty days after pancreatectomy, the dog was playful and appeared normal, with the exception that his

weight was below normal and the persistence of polyuria and polyphagia. On stopping the insulin injections his condition became rapidly worse, losing 4 kgm. in 20 days. He died in coma.

Dog 6 resembled dog 5 in that both lost weight at first in spite of insulin injections. After 3 to 4 weeks, there began a gain in weight, continuing as long as insulin was given, until a certain level was reached. This level, which was considerably below the original weight, was always lowered rapidly as soon as the insulin was discontinued. Several periods of no insulin were tried with similar results. After keeping the dog 200 days following the pancreatectomy, we stopped the insulin administration. The loss in weight was at first rapid and after a time more gradual. Forty-three days after discontinuing insulin the dog was very feeble, but still able to walk, and the appetite good.

The two dogs which we were able to keep in good condition, apparently indefinitely with insulin, each had a small piece of regenerated pancreas tissue at autopsy. The pancreas had, apparently, regenerated from the duct. That is, in both cases the pancreas was attached to the duct distal to the point at which the duct had been ligated and the portion distal to the ligature removed with the pancreas. In both cases the tissue was covered by the serosa of the duodenum. The smaller piece of pancreas was lost by accident. The larger piece from dog 6 contained some islets of Langerhans and appeared like normal pancreas tissue. The other four dogs, which lost weight continuously to the end, did not reveal a trace of pancreas at autopsy. However, in the cases of these four dogs continuous administration of insulin was not started until a marked degree of emaciation had developed.

Polyuria in absence of urine sugar. Even when the urine of the diabetic dogs was kept sugar-free by insulin there was still a marked polyuria. There was a reduction in the amount of urine excreted to about one-half the amount eliminated, when no insulin was given, but the daily urine volume in the well controlled dogs was more than twice the normal.

Polyphagia. The ravenous appetite which is so characteristic of depancreatized dogs was not affected by insulin treatment. All the dogs except dog 6, which was kept the longest, showed a marked polyphagia in spite of their urine being kept sugar-free. Dog 5 manifested the same degree of polyphagia after he had regained much of his weight as when he was very emaciated and was not receiving treatment. Dog 6 had a good appetite at all times, but he never ate greedily or such excessive amounts of food as did the other dogs.

The above facts—failure to maintain life by insulin in the absence of fragments of pancreas, persistence of polyphagia, and polyuria despite control of the blood sugar—seem to raise the question whether insulin is the entire pancreatic hormone. If insulin and the internal secretion

of the pancreas are identical, we would expect all the symptoms, which we ascribe to the lack of the pancreatic hormone, to be relieved. When dogs 5 and 6 had on insulin regained much of their original weight, there was a return of the sexual desires, which we have never observed in a depancreatized dog not receiving treatment.

Possible chronic toxic effect of our purest insulin. The appearance of some of the organs, in the dogs under insulin treatment for 100 days or longer, seems to indicate a possible toxic action of insulin. Since this was observed in only two cases it requires confirmation. The arteries and heart valves of dog 6 were extremely sclerosed. Such a condition had never been observed in a dog by those making the autopsy. The dog was between one and two years of age In the case of the liver there was a moderate enlargement. Microscopic examination revealed that fatty degeneration involved the entire liver. No normal liver tissue seemed to be present. Large calcium deposits were seen in the walls of the arteries. The alveoli of the thyroid were filled with colloid. The regenerated pancreas tissue appeared normal and the cells were filled with zymogen granules. There were a few islets of Langerhans, but not nearly as many as are usually found in a normal dog's pancreas.

CONCLUSIONS

1. Some totally pancreatectomized dogs may be kept alive and in fairly good nutrition for at least eight months on insulin. The normal body weight cannot be maintained, and even when the insulin doses are so regulated that little or no sugar appears in the urine, the polyphagia and some polyuria are present.

2. Some totally pancreatectomized dogs on insulin lose weight and die, despite the insulin control of the hyperglycemia and the glycosuria.

3. The determining factor in the temporary or indefinite survival of pancreatectomized dogs on insulin appears to be capacity of the duodenal stump of the pancreatic duct to regenerate pancreatic tissue, as such regeneration appeared in the two dogs surviving indefinitely, or at least up to 8 months, but not in the other dogs.

4. Insulin probably does not represent the entire pancreas hormone complex, since in the total absence of the pancreas insulin cannot maintain life or does not control all the diabetic symptoms (subnormal weight, polyphagia, polyuria).

5. The marked degeneration of the liver, and the extreme arterial sclerosis developed in dogs on long insulin management indicate either diabetic processes not controlled by insulin, chronic toxic action of insulin itself, or such toxic action of the other substances in the insulin mixture.

I wish to take this opportunity to express my indebtedness to Dr. A. J. Carlson for his very helpful suggestions in the course of this work.

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